HOST-PARASITE INTERACTIONS OF *COTYLURUS FLABELLIFORMIS* (FAUST,1917) (TREMATODA:STRIGEIDAE) IN NORMAL AND ABERRENT MOLLUSCAN HOSTS

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about our natural world and all of its mysteries

ABSTRACT

Life history studies were conducted concurrently on the hermaphroditic gastropod Lymnaea stagnalis appressa (Say) and the avian strigeid trematode Cotylurus flabelliformis (Faust) in the headwaters region of the Mississippi River. Information is presented on habitat characteristics, the seasonal population dynamics of the host-parasite system, aspects of intraspecific and interspecific trematode antagonism, and untoward effects that parasitization may have on the intermediate hosts.

In this habitat L. stagnalis was found to have an annual life cycle, although variation was evident and may serve a protective function in survival. Year to year variation was seen in the numbers of snails infected with C. flabelliformis sporocysts. The numbers of tetracotyles recovered per snail increased at a rate dependent on snail size. The ability of some adult snails to over-winter with these infestations is necessary in maintaining a stable parasite population.

The presence of C. flabelliformis, schistosome, or xiphidiocercariae-producing sporocysts in L. stagnalis were all associated with significantly smaller numbers of tetracotyles than in sporocyst-free snails. Conversely, tetracotyles did not develop in planorbid snails unless larval trematodes of other species were present. Biomphalaria glabrata proved to be an interesting exception in that sporocyst-free as well as schistosome-infected snails were highly susceptible to the penetration and development of these strigeid cercariae. The presence of sporocysts appears to be a limiting factor for tetracotyle development in lymnaeids, and a required factor for their survival in most planorbids.

While differences in growth between sporocyst-infected and metacercarial-infected snails were not apparent, significant mortality did occur when individuals were exposed to large numbers of C. flabelliformis cercariae in a short time period. The importance of this process as a mortality factor in nature, however, appears to be limited.

INTRODUCTION

Although much of parasitological research has in recent years moved into biochemical and immunological investigations, many details of life history phenomena of both host and parasite remain unresolved. Knowledge of these details is necessary to establish the dynamic nature of the host-parasite relationship, and to understand how the parasite population is regulated. The control of parasitic diseases for medical, veterinary, or economic reasons is based upon the results of ecological studies which identify those factors capable of influencing disease transmission.

Many of the principles of parasite population dynamics have come from the examination of non-human host-parasite systems which are readily accessible to most investigators. One such system involves Lymnaea stagnalis appressa (Say), a common freshwater pulmonate snail from lakes, streams, and ponds found over much of North America. This species is the intermediate host for several species of non-human schistosomes including Schistosomatium douthitti and Trichobilharzia ocellata, causative agents of cercarial dermatitis or "swimmer's itch" (Cort, 1950). Elsewhere, lymnaeid snails serve as vectors for fasioliasis of man and domestic animals. Numerous other trematodes infecting many wildlife species also utilize this family of snails.

In parts of North America L. stagnalis is found to be infected with Cotylurus flabelliformis (Faust), a strigeid trematode of waterfowl which utilizes this snail as both first and second intermediate hosts. The life history of this parasite is well matched

to that of its snail host, and to the migratory habits of its final hosts, usually anseriforme fowl of the family Anatidae. The relatively common occurrence of these two organisms provides ample opportunity for conducting seasonal studies on the population dynamics of both.

Previous studies into the biology of L. stagnalis have been reviewed by MacDonald (1969). More recently, Baxter (1977) studied the natural history of this species in a Minnesota lake. Because of the considerable infraspecific interpopulation variation noted in the course of the life cycles of freshwater pulmonates (Hunter, 1978), growth rate and life cycle studies on L. stagnalis were undertaken. This information also provides the necessary background to understand seasonal changes occurring in the parasite population.

The greater part of the life cycle of C. flabelliformis is spent within the intermediate hosts (adult worms survive only a matter of days in the definitive host). Seasonal studies on population changes of this parasite occurring in molluscan hosts are limited to those of Campbell (1972, 1973). Investigations into the prevalence and intensity of infection of intermediate hosts were undertaken to further evaluate seasonal changes and the degree of interpopulation variation which may occur.

Detailed examination into the natural history phenomena of this host-parasite system provided additional opportunities to study specific parasite-parasite and host-parasite interactions. These studies could be pursued in the laboratory because of the relative ease with which both species can be maintained.

The utilization of a single snail species for two different stages in the life history of C. flabelliformis raised interesting questions concerning "hyperparasitization" of the host. Winfield (1932) found that L. stagnalis harboring sporocysts of this parasite became ". . . highly resistant to the penetration of the cercariae of this species, which normally penetrate into and develop in uninfested snails . . ." Nolf and Cort (1933) promptly confirmed this, and also found evidence of a partial non-specific immunity to C. flabelliformis cercariae when snails were infected with the larval stages of a schistosome, Schistosomatium douthitti. The phenomenon may be considered a type of intraspecific trematode antagonism (interspecific in the case of S. douthitti), and has been demonstrated in several other species of strigeid trematodes. This study has presented further information into the nature of these trematode-trematode interactions.

Pathological effects that helminths have on molluscan hosts have been reviewed by Wright (1966) and include alterations in growth rate, size, reproduction, and mortality. Wesenberg-Lund (1934) commented on the exceptional size achieved by parasitized specimens of certain species of Lymnaea. The large amount of material examined in this study provided size comparisons between sporocyst-infested and sporocyst-free snails. Cercarial penetration and metacercarial encystment were demonstrated to be mortality factors in second intermediate hosts of certain echinostome trematodes (Kuris and Warren, 1980). The role that C. flabelliformis cercariae may play in mortality of host and non-host snails was evaluated in a similar

fashion.

This study presents information on a variety of topics concerning the biology of the host-parasite relationship. Specifically, investigations center on the seasonal population dynamics of the C. flabelliformis-L. stagnalis system. The importance of environmental (habitat) characteristics in relation to the success of the molluscan host (and consequently the parasite) is stressed. The pattern of infection in intermediate hosts provides the opportunity to explore aspects of intraspecific and interspecific trematode antagonism. Finally, both field and laboratory studies attempt to address the effects that parasitization may have on snail growth and mortality.

LITERATURE REVIEW

NATURAL HISTORY OF *L. STAGNALIS APPRESSA* (SAY)

Distribution:

Lymnaea stagnalis is a large pulmonate snail of Holarctic distribution occurring over most of Europe, Asia, North America, and the western-most part of North Africa. While absent on Greenland and Iceland, this species has been secondarily introduced into New Zealand (Hubendick, 1951). *L. stagnalis appressa* (Say) is the form most commonly found in North America and varies only slightly in morphology from that of its Eurasian counterpart, *L. stagnalis* (L.) (Hubendick, 1951).

Members of the genus *Lymnaea* are found in a wide range of habitats, but their common occurrence in isolated and small bodies of water most probably results from translocation via water birds (Hubendick, 1947). These snails are usually found on vegetation in shallow water and possess tenacious mucus, making possible adherence to the feet and feathers of birds with which they come in contact. As with most pulmonates, *L. stagnalis* is hermaphroditic, permitting colonization from one specimen. Both of these reasons help explain the snails' wide geographic distribution. Certain environmental conditions are necessary for growth and reproduction, and include clean water, suitable temperature range, living space, and the presence of extractable calcium (See MacDonald, 1969, for review).

The distribution and abundance of freshwater pulmonate snails are also felt to be strongly influenced by the concentration of

dissolved calcium in the aquatic environment. In fresh waters dissolved calcium is known to vary over 200-fold, and in those waters supporting molluscs with calcareous shells, more than 100-fold (Hunter, 1964, 1967). Generally speaking, extremely soft waters (those with less than 3 mg/l Ca^{++}) contain 5% of species and few individuals, whereas moderately soft waters (3-10 mg/l Ca^{++}) support about 40%. Intermediate (10-25 mg/l Ca^{++}) and hard (those greater than 25 mg/l Ca^{++}) waters contain up to 55% of the aquatic molluscan species of a region (Boycott, 1936; Hunter, 1964, 1978).

Boycott (1936) and later Macan (1950, 1963) found that the calciphile lymnaeids were virtually absent from waters containing less than 20 mg/l Ca^{++}. Attempts were also made in these studies to relate molluscan distribution directly with pH, but a more direct correlation was found with calcium content or total alkalinity. On the basis of water analyses and observations in southern Sweden, Hubendick (1947) concluded that ". . . the supply of calcium is the most important positive ecological factor for limnic gastropods. In the case of a poor supply of calcium, acidity appears to be the main negative factor." Other studies, showing the correlation of calcium content (or total alkalinity) with the distribution of L. stagnalis and Bulimnea megasoma, agreed with these findings (Tucker, 1958; Gilbertson, Kassim, and Stumpf, 1978).

Physiology and ecology:

While recognizing the importance of calcium to growth and reproduction, some disagreement has existed in the past regarding its

source as utilized by molluscs. Various studies have shown that a large proportion of the calcium requirements were fulfilled by direct absorption from the external milieu (Tucker, 1958; van der Borght, 1963; Greenaway, 1971; Wilbur, 1972; Thomas & Lough, 1974; Thomas, Benjamin, Lough & Aram, 1974). A smaller portion of these requirements (up to 20% in L. stagnalis) may be satisfied by absorption from food (van der Borght and van Puymbroeck, 1966).

While the previously mentioned studies have described the importance of calcium as it related to the distribution of aquatic pulmonates, there were fewer accounts correlating environmental calcium levels with growth characteristics. Thomas & Lough (1974) and Thomas, Benjamin, Lough and Aram (1974) have described the association between calcium concentration and growth in Biomphalaria glabrata. These authors reported that both absolute and specific growth rates tended to increase when volume was kept constant and calcium concentration increased. The net uptake rate of calcium from the external medium correlated significantly with the growth rate of these snails. Culture experiments with B. pfeifferi (Frank, 1963), and sampling of natural populations of L. peregra (Hubendick, 1947; Hunter, 1967) agreed with these findings.

Although both lymnaeids and planorbids appear to correlate growth rate and water hardness positively, some freshwater species have populations which differ significantly from each other in the extent to which they concentrate calcium from the environment. The calcium content in the shell of the freshwater limpet Ferrissia rivularis, for example, does not vary in a simple direct relation with

the amount of environmental calcium available (Hunter, 1967). L. stagnalis would be expected to show a correlation similar to that of other lymnaeids although, to this author's knowledge, there are no published accounts that have addressed this issue.

Numerous investigations into the growth rates and reproductive ecology of temperate freshwater pulmonates have been published (see Wilbur, 1964, and Fretter, 1978, for reviews). Considerable infraspecific variations in these parameters are known, and can be largely explained on the basis of environmental differences between habitats, with a smaller genetically determined component (Hunter, 1961a). Variation from year to year in mean adult size and population density of selected locations may reflect weather conditions of the previous year (Hunter, 1961b). Differences in other environmental conditions such as water hardness (previously discussed), temperature, and nutrition all contribute to interpopulation variation.

Being metazoans, molluscs are subject to the direct effects of temperature on metabolism and active transport reactions. Below a given temperature, feeding activity and locomotion cease and growth becomes negligible (Wilbur, 1964). Each species has a characteristic optimal temperature range for maximum growth and reproduction. Work on Physa pomilia and L. columella Say by DeWitt (1967) concluded that initiation of egg production was a function of temperature rather than day-length. Vaughn (1953) found that at 24°C more eggs of L.stagnalis appressa were produced and hatched than at any other temperature. No eggs developed or hatched below 9.9°. Vaughn stated that the "biological zero" (lack of growth) for L. stagnalis appressa was

around 11°C, and concluded that the optimum temperature for the growth and development of this species lay somewhere between 16° and 20°C. Van der Schalie (1973) later found 18°C to be the optimal temperature for growth in L. stagnalis, and 24°C optimal for reproduction, although survival at this higher temperature is slightly lower than at 18°C.

Recent works by Eisenberg (1966, 1970) and Baxter (1977) have concentrated on the influence that food supply and population density have on regulating population dynamics of L. elodes and L. stagnalis, respectively. Both of these studies have shown fecundity and growth to be density dependent and resource limited. When snail populations at high densities were provided with excess food, reproduction was still inhibited relative to low density populations. Baxter felt that some additional factor (an unidentified excretory product) other than food limitation contributed to the reduction of fecundity in dense populations of these snails.

Life cycle:

The literature on the normal life span of various pulmonates showed significant disparity (see Table 1) in life cycle descriptions. Baker (1911) stated that lymnaeids reach maturity in about two years, and could survive for up to four years. Boycott (1936) believed that all pulmonates in Britain had an annual cycle and survived for up to fifteen months. Crabb (1929) and Noland and Carricker (1946) found the maximum span of life for L. stagnalis in the laboratory to be nine and fourteen months respectively. Berrie

TABLE 1. Recorded Life Spans for Selected Pulmonates

Mollusc	Life Span (months)	Reference
Pulmonata	9-15	Boycott, 1936
Lymnaeidae	36-48	Baker, 1911
L. stagnalis L.	12-24	Berrie, 1965
L. stagnalis appressa	14	Baxter, 1977
L. stagnalis appressa	12	Noland and Carricker, 1946
L. stagnalis appressa (lab reared)	14	Noland and Carricker, 1946
L. stagnalis appressa (lab reared)	9	Crabb, 1929
L. elodes	12	Eisenberg, 1966
L. palustris	12	McCraw, 1970
L. palustris (lab reared)	8-10	Forbes and Crampton, 1942
L. peregra	12-14	Young, 1975
L. humilis	4-9	McCraw, 1961
F. modicella	4-8	VanCleave, 1935
F. parva	12-13	Hoff, 1937
L. haldemani	12	Morrison, 1932
B. megasoma	12-14	Gilbertson et al, 1968
P. gyrina	12	DeWitt, 1955
P. fontinalis	12	Duncan, 1959
A. fluviatilis	12	Hunter, 1961

(1966) described a life cycle for L. stagnalis in Scotland that could extend over two years in unfavorable habitats. Because the snails in that study did breed in their first year, Berrie felt that Boycott (1936) was justified in regarding L. stagnalis as an annual. Apparently, the life cycle was extended to include a second year when environmental conditions deteriorated.

Studies in Minnesota on L. stagnalis by Baxter (1977) suggested a 12-14 month life cycle in which snails were born in late summer and fall, overwinter, resumed growth in spring, and attained sexual maturity with reproduction in late summer and fall. Most of the adults died in the fall, although a small number overwintered a second time and bred again the following spring. Baxter felt that these overwintered adults played an insignificant role in the population dynamics of L. stagnalis, at least in Horseshoe Lake. Several species of related lymnaeids, including L. stagnalis (L.) (Berrie, 1965), L. humilis (McCraw, 1961) and B. megasoma (Gilbertson, Kassim, and Stumpf, 1978) also have an annual life cycle, but unlike that described by Baxter, reproduction in these species occurs primarily in spring and early summer.

As is evident in the studies just mentioned, considerable variation in life cycles occurs with natural populations of temperate freshwater pulmonates. Russell Hunter (1978) reviews the seven principal patterns of life cycles in temperate populations, and describes a simple univoltine pattern with breeding most commonly occurring in late spring or early summer. The other annual pattern is characterized by late summer breeding. Most of the species

discussed above fit into one of these two categories. A biennial pattern with each generation capable of reproducing in two successive years fits the pattern described by Berrie (1965) for L. stagnalis (L.) in the west of Scotland.

NATURAL HISTORY OF *COTYLURUS FLABELLIFORMIS* (FAUST, 1917)

Systematics:

The Strigeidae are a family of digenetic trematodes which involve at least three hosts in the life cycle. The first intermediate host is always a mollusc, but the second intermediate host may be a snail, fish, or frog. The metacercariae are of the tetracotyle, diplostomulum or neascus type, and mature to adults in the intestine of birds and mammals. The body of the adult is divided into distinct forebody and hindbody regions. The forebody is cup-shaped and contains the oral and ventral suckers, pharynx, and tribocytic organ. The latter structure consists of a pair of lobes which provide for attachment to the host's intestine. The cylindrical hindbody contains the reproductive organs.

Dubois in *Synopsis des Strigeidae et des Diplostomatidae (Trematoda)* (1968) recognizes twelve species of *Cotylurus* and six subspecies (Table 2). The significant diagnostic and life history information on these species is presented in that work. McDonald (1969) provides an extensive summary into the life cycles, known hosts, and geographic distribution of the helminths of anatid waterfowl.

Faust (1917) first described *Tetracotyle flabelliformis* in material from the Bitter Root Valley, Montana. Cort (1917) and Cort and Brooks (1928) described *Cercaria douglasi* from *Physa parkeri*, *Stagnicola emarginata angulata*, *Lymnaea stagnalis appressa*, and *L. stagnalis perampla* found in Douglas Lake, Michigan. Van Haitsma

TABLE 2. Species and Subspecies of *Cotylurus* Recognized by Dubois (1968).

C. brevis

C. cornutus

C. erraticus

C. flabelliformis

C. raabei

C. strigeoides

C. syrius

C. cumulitestis

C. intermedius

C. pileatus

C. platycephalus platycephalus

C. platycephalus communis

C. gallinulae gallinulae

C. gallinulae hebraicus

C. gallinulae ban

C. gallinulae vitellosus

(1930) later proved that adult strigeid trematodes of the genus Cotylurus Szidat, 1929 develop from Tetracotyle flabelliformis Faust, 1917 which in turn developed from Cercaria douglasi Cort, 1917. The specific name became Cotylurus flabelliformis, taken from the first described stage of the life cycle. Subsequent investigations by Olivier and Cort (1941) revealed that the cercariae previously identified as C. douglasi actually represented two distinct but closely related species: one of which developed only in members of the family Physidae (C. douglasi). The other developed only in members of the family Lymnaeidae, and was shown by van Haitsma to be the cercariae of Cotylurus flabelliformis (Faust, 1917).

Life cycle:

The life cycle of C. flabelliformis has been studied intensively by various authors (Buscher, 1965, 1966; Campbell, 1972, 1973; Cort, Olivier, and Brackett, 1941; Cort, Brackett, and Olivier, 1944; Cort, Brackett, Olivier and Nolf, 1945; Ulmer, 1957; van Haitsma, 1931) and is summarized here. Adult worms mature in the intestine of wild and domestic anseriforme hosts (most commonly blue-winged teal, mallards, and lesser scaup) and are capable of producing eggs within 48 hours following their ingestion as metacercariae. The worms are short lived, and expelled from the intestine in 7-10 days (Campbell, 1973). Van Haitsma (1931) and Acholonu (1964, 1965) successfully infected chicks (Galliformes) in addition to ducklings. Campbell (1973) has added experimental hosts representing three new avian orders to this list: the pied-billed grebe (Podicipediformes), American coot

(Gruiformes), and the song sparrow (Passeriformes).

The eggs are expelled with the feces, and embryonation occurs in the water, the rate of development being dependent upon the temperature. Approximately three weeks at 23°C are required for C. flabellifomis (Table 3). After hatching the miracidia remain infective for several hours and readily penetrate the first intermediate host, usually L. stagnalis or Stagnicola emarginata. Development of the mother sporocyst proceeds with production of daughter sporocysts which migrate to the hepatopancreas. Here they grow into a tangled mass of slender vermiform bodies which produce the typical pharyngeate furcocercous cercariae (see Cort and Olivier, 1941, and Hussey, Cort and Ameel, 1958 for details on early developmental stages of Strigeids). The time required from miracidial penetration to emergence of cercariae is about six weeks (Table 3). Campbell (1973) states that emergence of cercariae is temperature and light dependent, and that one specimen of L. stagnalis can on the average shed over 16,000 cercariae per day.

Cercariae remain active swimmers for 4-5 hours. During this time they appear to locate molluscan hosts by chemoattraction (Campbell, 1972; Matovu, 1974) and readily penetrate those with which they come in contact. Principal snail hosts include L. stagnalis, S. emarginata, and closely related lymnaeids as well as certain leeches of the genus Helobdella (Campbell, 1973). Within the snails the cercariae migrate to the hermaphroditic gland (ovotestis) where they undergo a peculiar body reorganization to the tetracotyle stage known as holometabolic metamorphosis. Several accounts of this process

TABLE 3.

Stage Specific Developmental Times of Selected Strigeidae

Species Reference	Ova Embryonation		Miracidia to Cercariae		Cercariae to Tetracotyles	
	Days	Temp °C	Days	Temp °C	Days	Temp °C
C. flabelliformis (van Haitsma, 1931)	21	23	42	ST	42	ST
C. flabelliformis (Cort, Brackett, Olivier, 1944)	-	-	-	-	16-27	RT
C. flabelliformis (Campbell, 1973)	-	-	-	-	42+	12-14
"	-	-	-	-	18-24	22-24
"	-	-	-	-	12	30-32
C. erraticus (Olson, 1970)	15-16	24	35	20	14-21	21
C. lutzi (Basch, 1969)	22-28	28	25	25-27	17-18	25-27
C. brevis (Nasir, 1960)	21	19-24	-	-	23-45	19-24
S. tarda (Mathias, 1925) = C. brevis (Dubois, 1968)	45	16	42-56	20	42	ST
"	21	20	-	-	-	-
"	12	24	-	-	-	-
"	8	27	-	-	-	-
S. elegans (Pearson, 1959)	11	RT	26	RT	21*	HT
"	330+	8	-	-	-	-
D. flexicaudum (van Haitsma, 1931)	19	RT	45	RT	35-42	ST

RT - Room Temperature
ST - Summer Temperatures
HT - Host Temperature (may be warm-blooded)
* - An additional 14 days are required for cercariae to develop into mesocercariae at summer temperatures.

appear in the literature on this species and related strigeids (Szidat, 1924; Mathias, 1925; Harper, 1931; Wesenberg-Lund, 1934; Ulmer, 1957; Pearson, 1959; Nasir, 1960; Basch, 1969; Campbell, 1973). At first most all of the cercarial organs are broken down, and there is a marked increase in body size. The internal structures then undergo reorganization with the appearance of rudimentary structures for attachment (tribocytic organ and pseudosuckers in the forebody) and reproduction (hindbody) with a concomitant reduction in size. Eventually the tetracotyle stage is reached with its characteristic cyst wall. Cort, Olivier and Brackett (1941) established an arbitrary classification of these stages according to the developmental cnanges just mentioned which are, respectively, 1) "developing forms", 2) "pre-cysts", and 3) "cysts".

Development to the tetracotyle stage is dependent on temperature, species of host, and intensity of infection (or size of host). Campbell (1973) found this to be 18-24 days at 22°-24°C for L. stagnalis exposed to small numbers of cercariae (see Table 3 for species comparison). Infection of final hosts occurs when snails harboring mature tetracotyles are eaten. The tetracotyles remain infective for the life of the host.

Ecology of the host-parasite relationship:

Studies by Buscher (1965, 1966) and Campbell (1973a) have shown that the life cycle of C. flabelliformis was adapted to the migratory habits of the final hosts (primarily anatid waterfowl). Both of these studies revealed bimodal infection peaks with the highest percentages

occurring during the spring (April-June) and early fall (September). There was an almost total absence of adult parasites during mid-summer. In addition C. flabelliformis was not found in ducks at their wintering grounds on the Gulf of Mexico (Buscher, 1965). Because of the short life span of adult worms (7-10 days) infection only occurred while the hosts were migrating to or from their breeding grounds and passing through habitats containing the appropriate snail intermediate hosts infested with tetracotyles.

Surveys commenting on the incidence of larval C. flabelliformis infections have been performed (Acholonu, 1964; Anteson, 1970; Bourns, 1963; Cort, Hussey, and Ameel, 1960; Larson, 1961; Shaffer, 1971; Ulmer, 1955, 1957), but only Campbell (1973b) documented the seasonal changes occurring with the infection of lymnaeid snails by this parasite. Some of Campbell's results will be reviewed here to help understand the ecological relationship of this parasite with its intermediate hosts.

The emergence of cercariae showed a bimodal fluctuation with a small number of snails (3%) shedding during late spring (May-June), and a large peak (30-60%) shedding in late summer (August-September). Those snails shedding cercariae in late spring acquired the infections the previous year and successfully overwintered. The virtual absence of cercarial-shedding snails in mid-summer was explained by 1) the death of overwintered snails in May and June, and 2) insufficient time for patent infections to develop in young snails. The higher percentage of snails shedding cercariae in late summer resulted from the spring migration during which time a higher percentage of duck

hosts were infected with adults of C. flabelliformis. Campbell (1973b) believed that yearly fluctuations in water temperature and migration patterns accounted for the variability of dates seen in the peak of cercarial emergence in late summer.

The occurrence of tetracotyles also showed a bimodal pattern with the appearance of large numbers in the fall following the peak of cercarial shedding in August and September. These tetracotyles were infective to final hosts during the fall migration. Although most of the host snails died off in the fall, many did overwinter and provided infective tetracotyles for the spring migration. These overwintered snails accounted for the second (spring) peak of metacercarial infections. As these remaining snails died off in June few tetracotyles could be found in the snail population, as the new generation of snails were too young to harbor mature tetracotyles.

Most all of the parasite's life cycle (except for one week as an adult) was spent in the intermediate hosts. The importance of the life cycle of the snail host in the seasonal dynamics of the parasite is obvious. Campbell (1973b) summed up his interpretation of these seasonal changes occurring with C. flabelliformis: "All stages in the life history appear to be adapted to the migratory habits of the definitive hosts . . ."

INTERSPECIFIC AND INTRASPECIFIC TREMATODE ANTAGONISM IN THE SNAIL HOST

Antagonistic interactions between larval trematodes of two different species within single snail hosts have been studied intensively in recent years, both from an ecological viewpoint and as potential biologic control agents for the elimination of trematode infections of man and his domestic animals. Reports on naturally acquired multiple infections have provided information on the interactions that various species have within the same host. On the basis of infection rates for individual species in a specific locale, the expected frequency of multiple infections can be calculated. Bourns (1963) was able to demonstrate the predicted number of double infections of C. flabelliformis and S. douthitti in L. stagnalis appressa in Ontario. Infections with other species, however, were able to increase or decrease the mollusc's susceptibility to subsequent infection. Certain combinations of plagiorchid and strigeid sporocyst infections occurred far less commonly in nature than expected on the basis of chance (Cort, McMullen, and Brackett, 1937). Other studies (Anteson, 1970; Cort, Hussey and Ameel, 1960; Donges, 1972; Probert, 1966) have also demonstrated the rarity of multiple infections, implying that some type of antagonism prevented their occurrence.

Most publications have dealt with two types of trematode antagonism: direct and indirect. Predation by rediae against other trematode larvae (other rediae or sporocysts) forms the basis for direct antagonism. Infection experiments with various species of echinostomes, schistosomes, and Fasciola in both planorbid and

lymnaeid snails have demonstrated stage specific dominance of certain species over others (Lie, 1973; Lie, Basch, Heyneman, Beck, and Audy, 1968; Lim and Heyneman, 1972). Basch (1970) demonstrated that rediae of Paryphostomum segregatum prey upon and eliminate sporocysts of Cotylurus lutzi in Biomphalaria glabrata.

Indirect antagonism may occur between rediae and sporocysts, or between sporocysts The mechanism of this process is unknown but of particular interest because, in the absence of predatory activity by rediae, a direct form of antagonism between species cannot occur. Possible explanations for it include 1) elaboration of inhibitory substances by sporocysts, 2) a cellular or chemical reaction by the snail host, or 3) inter-trematode competition for nutrients. The latter explanation has support from the studies by Cheng and Lee (1971, 1972) and by Gilbertson, Etges, and Ogle (1967) in which levels of carbohydrate, total protein, hemoglobin and free amino acids in hemolymph of B. glabrata are shown to be significantly decreased following infection with S. mansoni. Similar changes in the hemolymph of Planorbarius corneus infected with Cotylurus cornutus are also reported (Stadnychenko, Ivanenko and Burgomistrenko, 1980).

Basch, Lie, and Heyneman (1969) have described sporocyst-sporocyst interactions between Cotylurus lutzi and S. mansoni. Double infection of B. glabrata with these two species usually resulted in the complete inhibition of the strigeid sporocyst and eventual release of only schistosome cercariae, no matter which parasite entered the snail first. Anteson (1970) was unable to induce double infections of C. flabelliformis and D. flexicaudum in L.

catescopium pallida. Whichever parasite entered the snail first would dominate the one entering later, implying equal dominance by the second-stage sporocysts over each other's first-stage sporocysts.

Another type of indirect antagonistic interaction was found to exist between the metacercarial stages of certain strigeid, echinostomatid, and brachylaemid trematodes and the sporocyst stage of the same or, occasionally, some other species. Those parasites that demonstrated this phenomenon utilized the same species of snail as both first and second intermediate hosts. Harper (1931) noted that lymnaeid snails harboring sporocysts contained few specimens of Tetracotyle typica compared to uninfested snails from the same locality. Winfield (1932) was the first to document that L. stagnalis harboring sporocysts of C. flabelliformis contained negligible numbers of metacercariae compared to uninfested snails which contained large numbers of the developing forms. These observations were confirmed by Nolf and Cort (1933) and Wesenberg-Lund (1934), who likewise agreed with Winfield that the infested snails became highly resistant to the penetration of the cercariae of this species. Nolf and Cort (1933) also found evidence of a partial non-specific immunity to C. flabelliformis cercariae when L. stagnalis were infested with sporocysts of S. douthitti. In this case significantly larger numbers of the developing metacercariae were present in control groups than in the schistosome-infested groups, and those in the infested snails were much smaller, showing a retardation in development. Cort, Brackett, Olivier and Nolf (1945) later demonstrated similar findings in snails infested with sporocysts and rediae of D. flexicaudum, P. muris, and

E. revolutum. The few cercariae that did penetrate these snails, however, developed normally as metacercariae within the germinal sacs of the other trematode species. Several other species of strigeid and plagiorchid trematodes did not display evidence of non-specific immunity, indicating that the reaction is species and not group specific.

The phenomenon that Winfield described is known to occur in several other species of trematodes as well: 1) Panopistus pricei (Brachylaimidae) (Krull, 1935); Postharmostomum helicis (Brachylaimidae) (Robinson, 1949; Ulmer, 1951); Cotylurus lutzi (Strigeidae) (Basch, 1969, 1970); and Echinoparyphium aconiatum (Echinostomatidae) (Matovu, 1974). Basch (1970) termed this aversion to penetration and development of cercariae in snails infested with sporocysts of the same species the "Winfield Effect." In a series of interesting experiments Basch found that if the sporocysts of C. lutzi in B. glabrata were eliminated by concurrent infection with predatory echinostome rediae, the snails subsequently became susceptible to cercarial penetration and metacercarial development by C. lutzi. The immune effect was present only as long as strigeid sporocysts were present. Once cleared of sporocysts by rediae, C. lutzi cercariae readily penetrated and developed. Basch described this effect as a type of premunition: "The barrier to encystment is likely to reside not in an immune reaction of the snail host but in some suppressive or inhibitory substance contained within the sporocysts, or the cercariae themselves . . ."

Various species of lymnaeid snails serve as second intermediate

hosts for C. flabelliformis. Cercariae of this strigeid do penetrate physid and planorbid snails in large numbers, but only develop in those harboring sporocysts or rediae of other trematode species (Cort, Olivier, and Brackett, 1941). The cercariae penetrate the germinal sacs of the other larvae and reach the tetracotyle stage more quickly than in the ovotestis of lymnaeids. In uninfested snails the cercariae do not develop and soon die. Cort, Brackett, Olivier, and Nolf (1945) speculate that once the cercariae have entered other larval trematodes they are protected from any immune reactions of the abnormal host, and as hyperparasites are able to utilize the nutrition provided by the sporocysts or rediae intended for their own progeny.

The complex relationships that exist between larval trematodes within the same snail host show significant variability. The interpretation as to actual mechanisms responsible for resistance likewise displays much disparity. Culbertson (1941) and Feng (1967) accept the "Winfield Effect" as a demonstration of acquired immunity. Rather than implicating immunity in its accepted sense, Wright (1966) attributes the failure of Cotylurus cercariae to penetrate a snail infested with its own sporocysts to some form of chemical repulsion. Basch (1970) agrees with this possibility and also suggests that the increased mucous production seen in snails infested with sporocysts may be contributory. Chemotaxis does play a role in host location by cercariae (Campbell, 1972) and may be negatively affected by pre-existing redial stages in the snail host of Echinoparyphium aconiatum (Matovu, 1974). The actual mechanisms remain unresolved.

PATHOLOGICAL EFFECTS OF PARASITES ON HOST AND NON-HOST MOLLUSCA

Much of the literature describing pathological effects that helminths have on molluscs deals with digenetic trematodes and pulmonate gastropods. Most all Digenea are obligate parasites of molluscs and many utilize more than one intermediate host before becoming infective to the definitive host. The larval forms of these trematodes share intimate contact with the organs and tissues of their hosts resulting in pathological damage, but the type and degree of damage varies tremendously depending on the stability and specificity of the host-parasite relationship. In regard to this latter point, molluscs are able to mount a strong cellular response to the presence of larval trematodes with an increase in the number of circulating hemocytes (Stumpf and Gilbertson, 1978, 1980), and often either phagocytosis or encapsulation (Jeong and Heyneman, 1976; Lie and Heyneman, 1975, 1976; Michelson, 1975; Pan, 1965; Probert and Erasmus, 1965). In addition, certain biologically active substances in molluscan hemolymph which appear following infection have been identified and may help mediate the cellular immune response (Tripp, 1975).

Pathogenicity is influenced by habitat characteristics, snail population density, intensity of infection, and localization of the parasite in the host, among other factors. Pathological changes are known to occur in the tissues, blood, and body chemistry of the host in addition to alteration in growth rate, size, reproduction, and mortality (see Wright, 1966, for review).

Wesenberg-Lund (1934) observed the effects that trematode

infestation had on Lymnaea: ". . . localities with heavily infected snails have remarkably large specimens. Infection causes excessive growth and size, not the opposite, as might perhaps have been expected." Rothschild (1936, 1941) and Boettger (1952) described similar findings in marine and freshwater prosobranchs, respectively. Oxyloma retusa parasitized with Neoleucochloridium problematicum collected by Kagan (1952) were found to be larger than uninfected snails. Lymnaea stagnalis infected with Trichobilharzia ocellata under laboratory conditions were found to grow more rapidly, reproduce little or not at all, and live for longer periods when compared with uninfected snails (McClelland and Bourns, 1969). Campbell (1973b) suggested that the rapid increase in average size of L. stagnalis seen in mid-summer (July) may be the result of gigantism caused by larval stages of C. flabelliformis.

Pan (1965) documented an increase in the growth rate of B. glabrata during the second to third week following infection with S. mansoni. By the seventh week however, the uninfected controls had overtaken the infected snails. Stunting has also been reported in Oncomelania quadrasi infected with S. japonicum (Pesigan, Hairston, Jauregui, Garcia, Santos, Santos, and Besa, 1958). Zischke and Zischke (1965, 1967) have found that Stagnicola palustris infected with E. revolutum grow more slowly than uninfected controls and were less fecund. Likewise, Ulmer (1951) found that when Anguispira alternata was infected with Postharmostomum helicis, ". . . growth of the snail host, once a sporocyst infection has become established, is severly retarded."

Explanations for the variability in growth seen in trematode-infected molluscs include both the direct and indirect effects that parasites may have on a host's digestive and reproductive organs. Stunting is known to coincide with the migration of daughter sporocysts to the digestive gland (Pan, 1965) and may be a trophic phenomenon. Zischke (1965) demonstrated the histological changes occurring in the hepatopancreas of stunted S. palustris infected with E. revolutum. There was reduction in the number of digestive gland tubules with degeneration and hypertrophy of those remaining, and decreased cell glycogen. In addition, L. stagnalis infected with digenetic trematodes were found to respire at a lower rate than controls (Duerr, 1967). Wright (1966) in contrast suggested that gigantism may result from pressure effects on the digestive gland which are relieved by increases in shell capacity. Others (Baudoin, 1975; Rothschild, 1941) suggested that a curtailment in the reproductive effort of the host is a parasite's adaptation giving rise to increased host suurvivorship and growth, thereby benefiting the parasite.

Garnault (1889), in studies on Helix aspersa, gave one of the first accounts of parasitic castration in molluscs by trematodes. Since then numerous reports have appeared about the inhibiting effects that larval trematodes have on molluscan reproduction (Etges and Gresso, 1965; Looker and Etges, 1979; McClelland and Bourns, 1969; Najarian, 1961; Persigan et al, 1958; Zischke, 1967). The actual mechanisms affecting the gonadal tissues are varied and include 1) the direct action of rediae, 2) sporocyst growth with pressure atrophy, 3)

blockage of gonadal blood vessels, 4) reduction or degeneration of terminal genitalia, 5) differential nutritional drain, and 6) idiopathic mechanisms resulting in decreased fecundity and egg vaiability (Wright, 1966).

Bourns (1974) has evaluated the carbohydrate and protein content found in eggs of L. stagnalis and cercariae of T. ocellata. The nutritional drain associated with egg production was found to be greater than that seen in cercarial production. McClelland and Bourns (1969) speculate that when the snail host is freed from reproductive activity, the differential nutritional drain may account for increased growth and longevity, thereby (in Baudoin's words from 1975) ". . . increasing the parasite's Darwinian fitness."

Mortality of host snails associated with trematode infection displays a variability similar to that seen with growth. Wesenberg-Lund (1934) commented on host mortality associated with infections: "If a species of snail in a pond or small lake had been strongly infected one year, the snails had almost totally disappeared the next." Most observations on field collections suggest that infected snails die more rapidly once in the laboratory than do uninfected individuals. Etges and Gresso (1965), and Olivier, von Brand, and Mehlman (1953) have shown that S. mansoni-infected B. glabrata are more sensitive to environmental changes than controls. Under favorable conditions, though, infected snails may survive a length of time approaching that of uninfected controls (Ritchie, Taubr, and Edwards, 1963). McClelland and Bourns (1969), however, state that ninety percent of T. ocellata-infected L. stagnalis can

survive past twenty-eight weeks, whereas all of the controls are dead by that time. Because of this variability there is no concensus as to the role parasitism plays in limiting the host population.

Those snails which act as both first and second intermediate hosts to trematodes are especially vulnerable to significant mortality from a variety of actions by the larval stages: 1) development of sporocysts and rediae, 2) emergence of cercariae, 3) penetration and migration of cercariae, and 4) development and encystment of metacercariae.

Penetration of host snails by large numbers of cercariae has been shown to result in significant mortality of the snails. Van Haitsma (1931) and Nasir (1960) described the high mortality associated with the experimental exposure of host snails to cercariae of the strigeids C. flabelliformis and C. brevis, respectively. Similar effects with echinostome cercariae were noted by le Roux (1953) and Bayer (1954), who suggested their possible role as biological control agents. Even the leech Herpobdella punctata known to be a normal host for Apateman gracilis died within a day or two after being exposed to excessive numbers of cercariae of this species (Willey and Rabinowitz, 1938; Stunkard, Willey, and Rabinowitz, 1941).

More recently, Kuris and Warren (1980) have investigated the roles that cercarial penetration and metacercarial encystment of Echinostoma liei in B. glabrata have as snail mortality factors. Growth and survival were seen to decrease when snails were exposed to increasing numbers of cercariae. Highest mortality occurred within two days after juvenile snails (3-8 mm) were exposed to 500 cercariae

each. Those snails which survived the first two days did not suffer further unnatural mortality, indicating that the processes of penetration and migration alone were responsible for the deaths of the hosts. The high rate of cercarial penetration required to significantly alter host mortality patterns casts doubt on the potential of this process as a method of biological control.

MATERIALS AND METHODS

LAKE CHEMISTRY

The Nicollet Lake habitat was investigated for the period of April 1975 to November 1977, beginning soon after ice-off and continuing until fall. The physiography and chemistry of Nicollet Lake were studied, and included measurements of the more common limnological parameters. Hydrogen ion measurements were made by a Beckman Model G pH meter; dissolved oxygen content was measured by the Winkler iodometric method; total hardness and Ca^{++} hardness were determined by the titrametric method using EDTA (A.P.H.A., 1971); total carbonate alkalinity and phenolphthalein alkalinity (CO_2) were determined by the titrametric method using 0.02 N H_2SO_4 with bromcresol green-methyl red and phenolphthalein respectively, as indicators (A.P.H.A., 1971).

FAUNA SURVEY

Sampling of L. stagnalis during this time usually occurred at two week intervals. Collections were made either by hand-picking or using a dip net, of all snails seen in the immediate vicinity of the collector before moving to a new area in the lake. All snails were collected within the limnetic zone as they floated on the surface or crawled along aquatic vegetation. By attempting to collect all snails within a given area before moving on, the inherent bias of the collector towards the selection of larger snails would be minimized. A goal of collecting at least 100 specimens per visit was set but not always adhered to because of changing weather conditions and variable

water turbidity. No snails over a depth of one and one-half meters were taken because of a minimum amount of turbidity always present. Before each sampling, water temperature at approximately 10 cm was also taken. All specimens were brought to the laboratory and measured from shell base to apex (altitude or height) to the nearest 0.1 mm, using either a dissecting microscope fitted with an ocular micrometer for the smaller snails or with vernier calipers for the larger ones. Snails were then placed individually into 4 oz. glass jars containing aged tap water and left under an incandescent lamp for approximately 18 hours. The jars were examined under a dissecting microscope for the presence of trematode cercariae which were subsequently stained with neutral red and identified according to body type.

QUANTITATION OF TETRACOTYLES

Those snails found to be shedding cercariae were dissected to free any additional larval trematodes. An additional number of non-cercarial shedding snails were also dissected. All snails of both groups (other than very young specimens) were found to contain numerous metacercariae of C. flabelliformis in various stages of development (see Ulmer, 1957). Of the three metacercarial stages described for this species, those which had reached the cyst or tetracotyle stage were quantitated by one of two methods: 1) a direct count of all tetracotyles found in the dissected specimen; or 2) an indirect count utilizing a dilution and averaging technique.

The latter method was devised for this study and was performed in several parts. First the digestive and hermaphroditic glands (which contain essentially all of the metacercariae) were dissected

free and teased apart. This material was washed into a Waring blender with a small amount of normal saline. The blender was activated at medium speed for only one second, to free any additional tetracotyles trapped within the tissue matrix. Next the contents were transfered to a 150 mL flask and diluted to a total volume of 100 mL. While the flask was gently agitated to evenly disperse the organisms, three 10 mL fractions were withdrawn and transferred to flat-bottomed watch glasses for counting. The three counts were then averaged and multiplied by ten for the final total count. Only mature metacercariae (tetracotyles) were counted by this method, and were easily distinguished from the developing forms. Although the developing forms were distorted by use of the blender, the tetracotyles appear untouched.

Five sample snails were evaluated for total numbers of tetracotyles using both methods. None of the results using the dilution and averaging technique varied more than 10% from the direct counts. Counts from all snails with greater than 500 tetracotyles each were made by this method.

MAINTENANCE OF LIFE CYCLES

Cultures of L. stagnalis originating from Nicollet Lake, and L. elodes from a prairie slough near Bejou in Mahnomen County, Minnesota, were grown in the lab for purposes of maintaining the intermediate stages of C. flabelliformis and S. douthitti, respectively. These snails were maintained in 5 gallon aerated aquaria containing aged tap water at both 18 and 24° C, with a twelve hour light/dark cycle. Food consisted of leaf lettuce and Purina rat chow with chalk added as an

additional calcium source.

Definitive laboratory hosts for C. flabelliformis included domestic Rouen ducklings supplied by Sabania Hatchery of Little Falls, Minnesota, and fryer chicks supplied by Jack Frost Hatchery of St. Cloud, Minnesota. Both ducklings and chicks were force fed tetracotyles which had either been dissected free or were still present within mollusc tissue. After several days (see van Haitsma, 1930 and Campbell, 1973) numerous trematode eggs were present in the feces. These ova were recovered by washing and sieving the fecal material repeatedly with large volumes of aged tap water, and allowing the debris to settle before decanting. Large numbers of ova could be concentrated in this manner.

Anteson (1970) describes a method for maintaining C. flabelliformis in the laboratory, but an easier method for mass infection of snails is described as follows: a thin layer of ova-enriched detritus is poured into a two and a half gallon aquaria and filled about two-thirds full with aged tap water. A stone bubbler is introduced to prevent stagnation. During the following weeks, ova can be examined periodically for miracidial development, and, at the appropriate time (2 weeks at 24° C in this study), young L. stagnalis (twenty 5-20 mm snails) are introduced into the tank. The snails are then maintained as described previously. Infectivity rates greater than 85% are obtained using this method.

Snails begin shedding cercariae after 4-6 weeks and, when placed with uninfected laboratory raised L. stagnalis, the cercariae are seen to actively attach to and penetrate the snail. Depending on the

length of time uninfected snails are exposed to cercarial shedding snails, large numbers of developing metacercariae and eventually fully formed tetracotyles will appear following sufficient time for development (18-24 days at 22-24° C according to Campbell, 1973). These tetracotyles are then infective for the final host.

Adult C. flabelliformis were recovered from ducklings or chicks 3-5 days after ingestion of tetracotyles. This was determined by sacrificing the bird host and examining the intestine. The adult worms recovered were fixed in warm AFA (alcohol-formol-acetic) fixative and stored in 70% alcohol. Staining with Semichon's acetic-carmine was performed and the specimens mounted with Permount. Permanent preparations of tetracotyles were prepared in the same manner. Measurements of these specimens were performed on a compound microscope fitted with an ocular micrometer.

Definitive hosts for S. douthitti included laboratory reared Peromyscus (deer mouse) and the white laboratory mouseMus musculus. The maintenance of this parasite's life cycle in the laboratory was very similar to that of Schistosoma mansoni. Mice were easily infected by exposure to a thin layer of cercarial-infested water in a flat-bottomed glass dish for thirty to sixty minutes . Cercariae for infecting the final host were collected by pooling L. elodes with patent infections and placing them in the dark for several hours, which stimulates cercarial shedding. The final concentration of cercariae was estimated by performing sample counts using a hemacytometer. This meant that semi-quantitative measurements of the number of cercariae presented to the host could be made. As with S.

mansoni, cercariae can be injected intra-peritoneally.

EXPERIMENTAL INFECTIONS

To examine molluscan host selectivity of C. flabelliformis, various species of gastropod and bivalve molluscs were exposed to varying numbers of cercariae and observed by dissecting microscope for attachment and penetration.

The effects that pre-existent trematode sporocysts had on the ability of tetracotyles to develop were determined in several ways: 1) careful dissections were made of L. stagnalis in which naturally acquired C. flabelliformis and other trematode sporocysts were present, and any tetracotyles found counted; 2) laboratory experiments were performed in which the following uninfected and infected snails were individually exposed to varying known numbers of C. flabelliformis cercariae:

1. uninfected L. elodes, B. glabrata, and H. trivolvis,
2. S. douthitti-infected L. elodes,
3. S. mansoni-infected B. glabrata.

All snails except for the H. trivolvis were lab-reared. These were collected from Nicollet Lake and examined periodically over several weeks for cercarial shedding. Only those H. trivolvis not shedding cercariae were used in these experiments. Following a length of time usually sufficient for tetracotyle maturation, all snails were dissected and counts of any metacercariae present were made.

The effect that cercarial penetration had on host mortality was examined by exposing snails (L. stagnalis, L. elodes, H. trivolvis and B. glabrata) to varying numbers of C. flabelliformis cercariae, and

observing subsequent die-off. Treatment groups, consisting of 20 snails each, were exposed to 100 or 500 cercariae per snail (each exposed individually) as a single exposure. Additional treatment groups were subjected to continuous cercarial exposure by having an individual C. flabelliformis-shedding L. stagnalis present in each tank throughout the experiment. Control groups for each species of mollusc used were also maintained to assess that mortality associated with the laboratory environment.

RESULTS AND DISCUSSION

ECOLOGICAL SETTING

Introduction:

Nicollet Lake was chosen for this study because of its isolated location and the large numbers of Lymnaea stagnalis found to inhabit the littoral area. The lake is located within Lake Itasca State Park in Clearwater County, Minnesota (T143N,R36W,S21-22) at an elevation of 1495 feet above sea level. The lake is fed by springs via an inlet at the south end and drained by an outlet on the west shore. This outlet shortly joins Howard Creek, the confluence forming Nicollet Creek which flows approximately 7300 feet with a drop of 20 feet in elevation into the west arm of Lake Itasca (Glazier, 1897). Although Lake Itasca is accepted as the source of the Mississippi, it has been said that ". . . if the highest source is sought, that honor would fall to Nicollet Creek . . ." (Schwartz and Thiel, 1954).

Geologic Setting:

The Lake Park Region in north and west central Minnesota which includes Nicollet Lake is characterized by the knob and kettle physiography of the Itasca moraine. This land form remained following the wastage of the Wadena lobe of the Keewatin ice sheet that covered much of north central Minnesota during the Hewitt Phase (Cary substage) of the Wisconsin glaciation, which ended approximately 40,000 years ago (Wright and Ruhe, 1965). Drift from this lobe of the ice sheet is generally of the clayey grey and calcareous type

(Zumberge, 1952) without Cretaceous shale, implying its origin in south-eastern Manitoba northeast of the area of Cretaceous bedrock (Wright and Ruhe, 1965).

The nature of this glacial drift is the single most important factor determining the characteristic chemistry, and type and abundance of aquatic life in these waters. The grey (Cary) drift as found in the Itasca area in north central Minnesota supplies an abundance of calcium carbonate and other nutrients to the lakes in this area. Thiel and Stauffer (1933) found this drift to be more permeable than later glacial drift, thus allowing for increased percolation of calcium carbonate containing ground water into the lakes. Most of these lakes have consequently been classified by Moyle (1945) as hardwater with total alkalinity ($CaCO_3$) ranging from 40 to 250 parts per million and summer pH from 8.0 to 8.8. Moyle (1949) later concluded that productivity was directly related to total alkalinity. The increased fertility as found in these lakes has resulted in high rates of sedimentation and eutrophication leading to senescence (Eddy, 1963).

Physical and Floral Characteristics:

Resting in the bottom of a probable pre-Pleistocene valley, Nicollet Lake fits the description given by Zumberge (1952) in his outline of lake classification on the basis of their origins as being of Type 3: ice block basins in outwash localized by pre-glacial valleys. Lake length is 288 meters north to south with an average width of 146 meters. Shoreline length is approximately 680 meters,

producing a surface area of 7.2 acres. Maximum depth is 6.7 meters.

The east and west banks of the valley are covered with a combination of red pine, balsam fir, white and black spruce, aspen, and paper birch. This woody flora is typical of the moist acid soils in coniferous forest regions, especially along streams and around lakes and ponds. Several feet above lake level on the hillsides, black spruce (Picea mariana) and tamarack (Larix laricina) are common. These give way to willows (Salix sp.) and finally to alders (Alnus sp.), the most common shrub surrounding the lake. Interspersed with occasional cattail (Typha sp.), sedges (Carex sp.) form an extensive mat of variable width around the lake. Wild rice (Zizania aquatica) and occasional pond-lilies (Nuphar sp.) comprise the band of floating plants and emergent vegetation. Submerged aquatics extend out to a depth of approximately 3 meters. These physical and biological relationships are summarized on the map of Nicollet Lake (Fig. 1).

Chemical Characteristics:

Chemical parameters of the lake were studied during May, 1973 by the author and several co-workers. Results have been shown in Table 4. Stratification into distinct epilimnial and hypolimnial layers was not apparent on temperature profiles because of the shallowness, small volume of the lake, and proximity of the inlet and outlet which all allowed for partial circulation and mixing. Dissolved oxygen determinations using the Winkler iodometric method demonstrated O_2 levels in the upper five meters to be relatively constant (averaging 8-12 ppm), but then approached zero near the bottom. This anaerobic

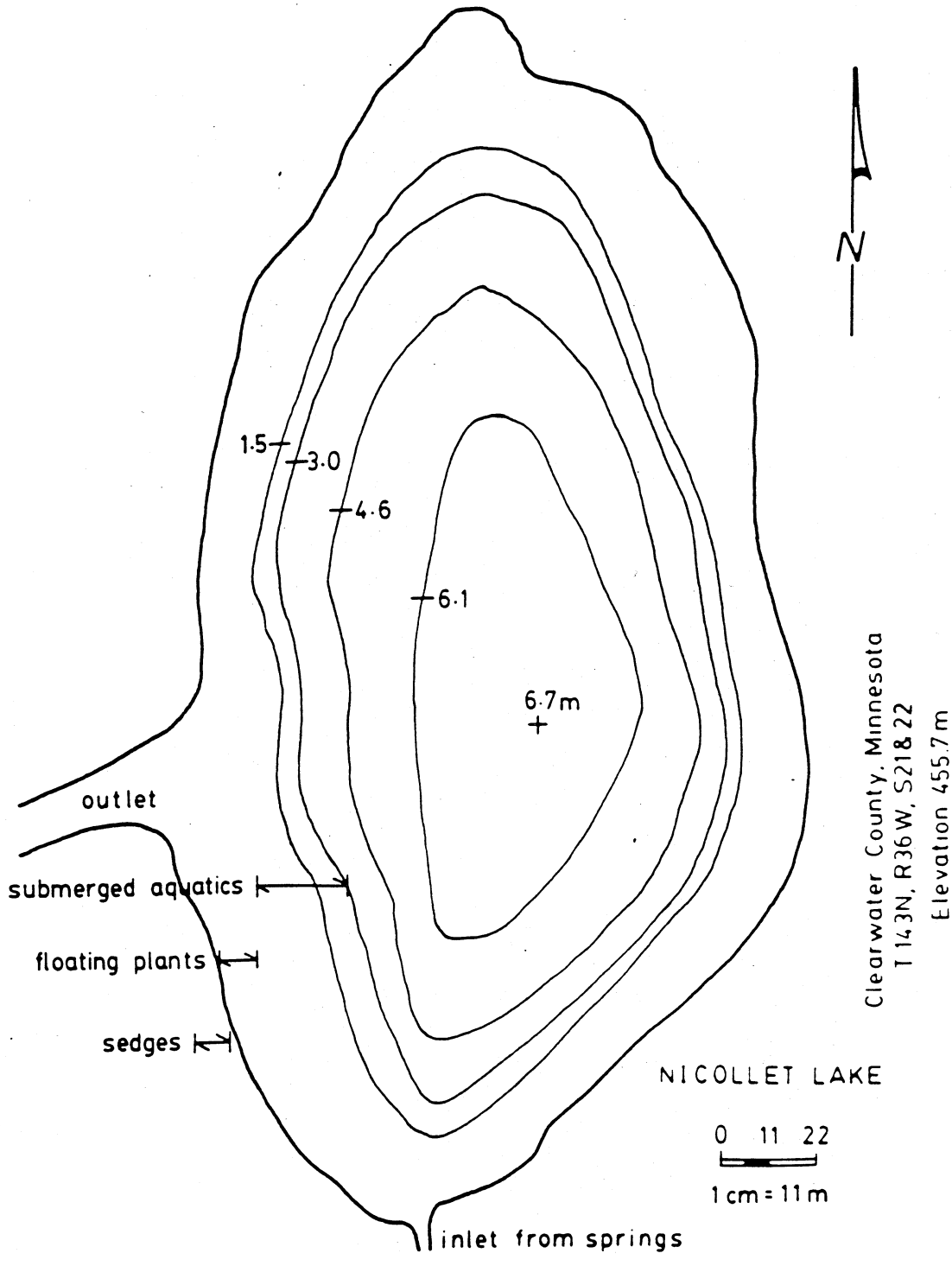

Figure 1. Physical characteristics of Nicollet Lake, Clearwater County, Minnesota compiled from measurements made during 1972 and 1973.

Table 4. Chemistry of water samples from Nicollet Lake, Clearwater County, Minnesota. Values reflect characteristic changes occurring over 24 hours. Maximum depth is 6.7 meters.

	Dissolved O_2 (ppm)	pH	CO_2 (ppm)	Total ($CaCO_3$) Alkalinity (ppm)	Ca^{++} Hardness (ppm)	Total Hardness (ppm)
1-5 meters	8-12	8.1-8.4	12-17	200-220	154	240
6 meters	< 1	< 7.5	> 35	> 240	154	240

condition corresponded with the type of methane and hydrogen sulfide producing autochthonous sediment found there: sapropel. Hydrogen ion concentrations remained constant in the upper five meters but decreased rapidly near the bottom where bacterial decomposition of detritus caused an increase in aggressive carbon dioxide content as was evident from the increase in phenolphthalein alkalinity. This titrametric method was a measure of $CO_3^{=}$ and OH^{-}. Total alkalinity (predominantly bicarbonate ion) as measured using the methyl orange indicator method also remained constant in the upper five meters, but then increased significantly near the bottom. This resulted from the formation of bicarbonate by the reaction of anaerobically produced CO_2 with $CaCO_3$. Ca^{++} hardness, and total hardness as determined by the titrametric method using EDTA, remained constant at all depths.

Fauna:

Fish, other than various small minnows, were unknown in Nicollet Lake. Shallowness and high anaerobic activity undoubtedly allowed for winter kill, preventing the establishment of more sensitive fish species. Water fowl and herons seen frequenting the lake include the following species in descending order of frequency: loons (Gavia immer), mallards (Anas platyrhynchos), blue-winged teal (Querquedula discors), great blue herons (Ardea herodias herodias), and American bittern (Botaurus lentiginosus). For several years the lake level varied because of the activity of beaver (Castor canadensis) in damming the stream outlet. Warm spring days inevitably saw the appearance of both painted (Chrysemys picta) and snapping turtles

(Chelydra serpentina). The aquatic molluscan fauna includes those species listed below:

Family	Species
Lymnaeidae	Lymnaea stagnalis appressa (Say)
Physidae	Physa gyrina (Say)
Planorbidae	Helisoma trivolvis (Say)
	H. campanulata (Say)
	Gyraulus parvus (Say)
	Promenetus exacuous (Say)
Valvatidae	Valvata tricarinata
Sphaeriidae	Sphaerium sp.

SPECIES IDENTIFICATION

Dubois (1968) and McDonald (1969) have recognized six species of Cotylurus occurring in anseriforme hosts in North America: C. brevis, C. cornutus, C. erraticus, C. flabelliformis, C. hebraicus, and C. strigeoides. Because of similarities often noted between closely related parasites, body measurements are often made to aid the morphological studies in species diagnosis. These measurements however, may also overlap, requiring the use of other criteria such as organ-specific morphology, flame-cell patterns in cercariae, and specificity of the parasites for intermediate and final hosts.

For purposes of identification, tetracotyles from several L. stagnalis, either dissected free or within snail gland tissue, were force fed to both chicks and ducklings. Chicks 2 and 1/2 weeks of age were refractory to infection whereas those 4 to 6 weeks of age began shedding numerous trematode eggs 4 to 6 days after exposure. Numerous adult strigeids identified as C. flabelliformis were recovered from the chicks at necropsy. Although the literature reflects some difficulty in infecting chicks compared to ducks, this has been accomplished by van Haitsma (1931) in chicks 6 to 7 weeks of age, Acholonu(1965) (ages unknown), and Campbell (1972) in 3 week old chicks. Domestic Rouen ducks were found to be easily infected at any age beyond six days (earliest exposure made). One of the ducklings died two days after being force-fed several adult L. stagnalis (shell and foot removed) containing several thousand tetracotyles. At necropsy the caecal wall was covered with adult worms and markedly hemorrhagic, undoubtedly accounting for the death of the host. Adult

worms from these ducks were also fixed in warm AFA and identified as C. flabelliformis.

Measurements of adult worms recovered from ducks in this study were compared with those of Campbell (1972) and van Haitsma (1931) for C. flabelliformis, and Dubois (1968) for C. brevis and C. cornutus (Table 5). Substantial overlap in many of the measurements of these species was obvious. Campbell (1972) performed extensive studies on the variation seen in measurements of C. flabelliformis raised in different avian hosts, and also the effects that differing types of fixation had on the same parameters. His studies showed considerable variation from those of van Haitsma (1931). The worms tend towards a larger size (depending on the host) which may reflect normal intraspecific variation, but Campbell also demonstrated that fixation with warm AFA results in the worms being more relaxed and fully extended (van Haitsma used cold fixative).

C. flabelliformis is one of the smallest species of this genus. The measurements presented in this study are essentially in agreement with those of Campbell and van Haitsma and fall somewhere between the two. While the lower end of the range for C. brevis does fall within the middle or upper ranges of C. flabelliformis, this species and C. cornutus are, for the most part, significantly larger. This fact, along with the distinctive testicular morphology of each, helps in identification.

A comparison of measurements between stained and mounted tetracotyles of C. flabelliformis (from this study and Hughes, 1928) and C. brevis (from Nasir, 1960) reveals the inherent difficulty in

Table 5. A comparison of the measurements of three anseriform *Cotylurus* sp. from preserved and stained adult whole-mount specimens (measurements in microns).

	C. flabelliformis Present Investigation Warm fixed			*C. flabelliformis* van Haitsma (1930) Cold fixed			*C. flabelliformis* Campbell (1972) Warm fixed		*C. brevis* Dubois (1968) Cold fixed	*C. cornutus* Dubois (1968) Cold fixed
	n	mean	(range)	n	mean	(range)	mean	(range)	(range)	(range)
Total Length	20	852	(700-1100)	10	716	(560-850)	950	(770-1120)	(1000-1800)	(1250-2750)
Forebody Length	20	347	(300-400)	10	239	(200-280)	370	(290-440)	(300-720)	(300-720)
Forebody Width	20	380	(300-460)	10	278	(220-320)	360	(280-440)	(300-540)	(340-800)
Hindbody Length	20	505	(380-720)	10	477	(360-570)	580	(430-680)	(540-1110)	(900-2100)
Hindbody Width	20	413	(320-500)	10	258	(200-260)	310	(260-400)	(260-660)	(380-740)
Ratio HB/FB Lengths	20	1.47	(1.10-2.00)	-	1.99	-	1.81	(1.40-2.30)	(1.25-1.94)	(2.43-4.40)
Oral Sucker Length	10	76	(70-90)	10	59	(50-70)	65	(10-76)	(72-120)	(65-155)
Oral Sucker Diameter	10	82	(68-92)	10	60	(40-80)	66	(59-78)	(61-120)	(65-140)
Pharynx Length	10	49	(42-54)	10	37	(30-45)	42	(35-49)	(50-59)	-
Pharynx Diameter	10	46	(40-56)	10	30	(20-40)	40	(33-49)	(36-45)	(45-110)
Acetabulum Length	10	91	(82-102)	10	70	(60-83)	86	(69-104)	(83-180)	-
Acetabulum Diameter	10	104	(90-110)	10	62	(40-80)	72	(10-104)	(66-170)	(100-200)
Ovary AP Diameter	10	77	(52-90)	10	51	(40-60)	76	(11-95)	(65-120)	(120-190)
Ovary DV Diameter	10	86	(70-106)	10	66	(50-75)	76	(64-99)	(75-150)	(125-220)
Tanned Eggs Length	55	100	(90-107)	10	107	(100-112)	108	(96-117)	(88-110)	(81-110)
Tanned Eggs Diameter	55	65	(55-80)	10	70	(68-76)	69	(64-74)	(50-70)	(51-73)

species identification at this stage (Table 6). Numerous publications describing the morphological characteristics of many species of Tetracotyle exist, but identification in most cases remains difficult. Considering that both C. flabelliformis and C. brevis utilize L. stagnalis as first and second intermediate hosts, the use of infection experiments with the identification of adult worms would still seem appropriate.

Table 6. A comparison of the measurements of Cotylurus flabelliformis and Cotylurus brevis tetracotyle metacercariae from preserved and stained wholemount specimens (measurements in microns).

	C. flabelliformis Present Investigation			C. flabelliformis Hughes (1928)			C. brevis Nasir (1960)		
	n	mean	(range)	n	mean	(range)	n	mean	(range)
Total Length	10	269	(225-306)	10	253	(218-282)	35	240	(200-264)
Maximum Width	10	212	(166-237)	10	191	(162-212)	35	244	(208-230)
Hindbody Length	10	72	(59-81)	10	81	(70-95)	35	72	(56-80)
Hindbody Width	10	136	(106-153)	10	133	(115-151)	35	120	(112-132)
Oral Sucker Diameter	10	43	(40-48)	10	47	(42-53)	-	-	(41-48)
Acetabulum Diameter	10	44	(40-49)	10	43	(35-49)	-	-	(46-55)
Holdfast Diameter	10	88	(66-103)	10	86	(77-98)	-	90	-

BIOLOGY AND GROWTH OF L. STAGNALIS APPRESSA (SAY)

Analysis of snail collections from Nicollet Lake for the years 1975 to 1980 were included in the appendix, Table A-1. Only the material for the years 1976 and 1977 was presented graphically as the sampling during those years was more complete, and provided a reference for comparing the collections made in 1975, 1978, and 1979. Shell altitude-frequency histograms for 1976 (Fig. 2) and 1977 (Fig. 3) showed similar changes occurring in the population in both years. Some variation in size was present when comparing similar dates in both years.

Soon after ice-off in both years, numerous young snails (2-5 mm) appeared at the water line attached to reeds. Large (43-57 mm) algae-encrusted overwintered adults were much less numerous, but by no means rare. Soon after these overwintered snails appeared in the lake, egg masses were noticed attached to reeds, and persisted as long as these large snails were collected. Within 36 hours after bringing these snails into the laboratory, numerous egg masses were also found on the sides of the aquaria.

Throughout spring the older snails showed only slight growth, whereas the young underwent an impressive growth spurt that peaked in mid-June. By the end of July, growth had almost stopped completely. During the next two and a half months (July 30-Oct. 16, 1977) the shell length sample mean increased slightly from 52 to 54 mm, although the water temperature usually remained above 16°C. Van der Shalie and Berry (1973) found 18°C to be the optimal temperature for growth in this species. Considering the lack of growth occurring at this time,

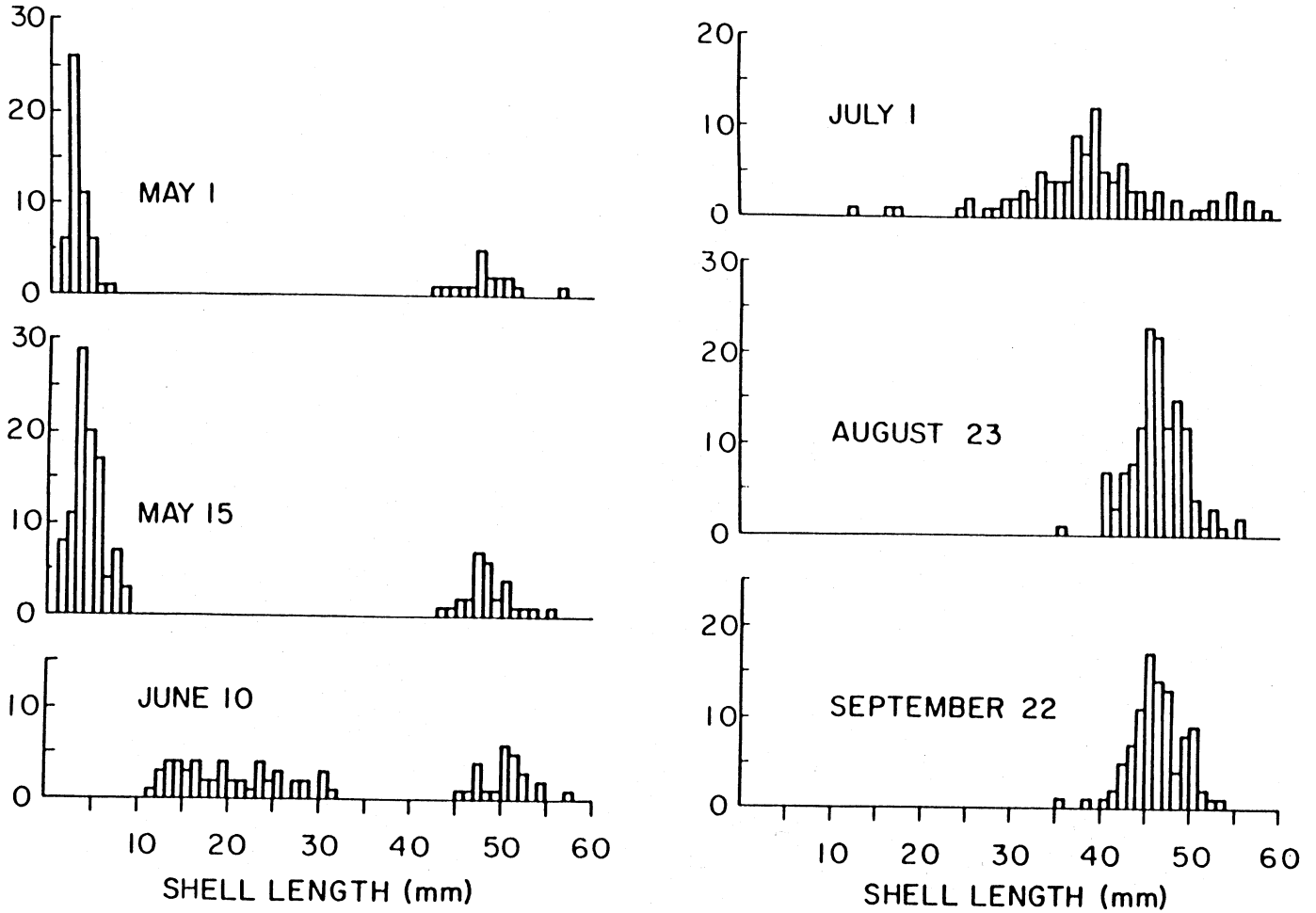

Figure 2. Altitude-frequency histograms of Lymnaea stagnalis appressa (Say) collected from May through September, 1976 in Nicollet Lake, Clearwater County, Minnesota.

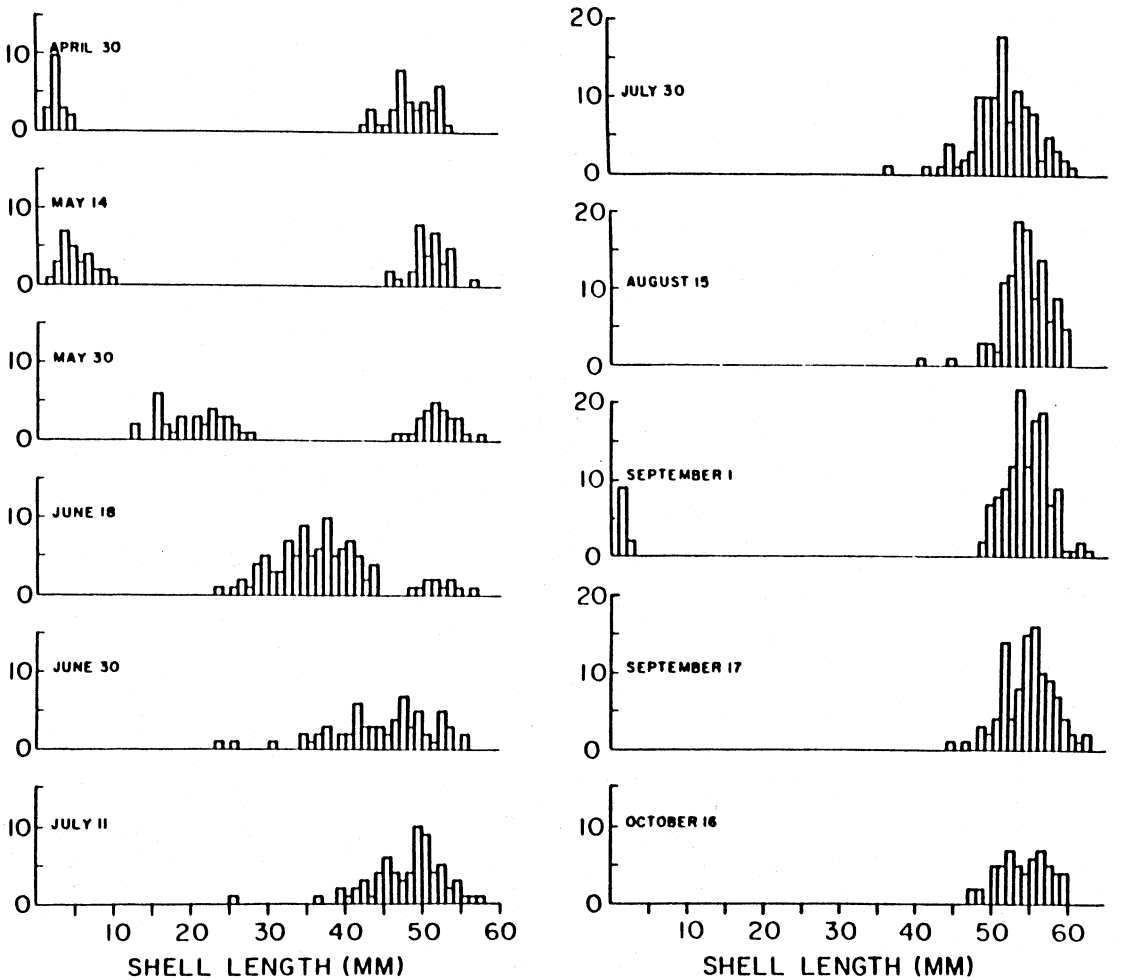

Figure 3. Altitude-frequency histograms of Lymnaea stagnalis appressa (Say) collected from April through October, 1977 in Nicollet Lake, Clearwater County, Minnesota.

it would appear that L. stagnalis does have some physiologic or genetic limit to the maximum attainable size.

By early July the older overwintered snails were no longer detectable in the collections, either as a result of differential mortality or incorporation, by size, into the rapidly growing younger population. The lack of callous-scarred (rest marked) or algae-encrusted specimens to differentiate age groups makes the former explanation more reasonable. In addition, many of these older snails were found dead floating on the water throughout the spring.

Egg-laying by the younger population became obvious in early August, with newly hatched (around 2 mm) snails appearing in the collection by early September. Although later collections did not include these newly hatched specimens, they were seen, along with egg masses, in the middle of September. No significant growth was apparent, however. One egg mass found on October 18, 1980 when the water temperature was 7°C remained viable when brought into the laboratory.

Decline in water temperature appears to explain the lack of growth occurring in the late summer-early fall hatch of snails. Studies by van der Schalie and Berry (1973) have shown that at 16°C newly hatched L. stagnalis achieve a length of only 3 mm after about 30 days. The temperature in Nicollet Lake in September varied from 15-17.5°C. The limited growth that can occur at this temperature was certainly slowed even more as the month progressed. By October water temperature was noted to be 7°C, well below the "biological zero" of this species (11°C) at which no growth occurs (Vaughn, 1953).

The young snails (2-5 mm) that appeared in the first collections in spring after ice-off undoubtedly originated from those hatched in late August and early September of the preceding year. Vaughn (1953) also reported that at 16°C, the eggs of L. stagnalis required about 59 days to hatch. This implies that egg masses laid in late summer (September) in Nicollet Lake would stand little chance of hatching or surviving that same year, *if at all, considering the high mortality at lower temperatures (60% at 14°C according to van der Schalie and Berry, 1973). Hence the burden of producing offspring capable of surviving until the next spring would fall on those snails reaching reproductive maturity in early and mid-summer* (21-27 mm according to van der Schalie and Berry, 1973). Fraser (1946) found that 2 weeks were needed for egg hatching in L. stagnalis at a summer temperature of 23°C. This provides ample time for the production of large numbers of progeny before the egg masses are inhibited from developing sometime in late summer.

During the middle of September, an annual phenomenon occured. This was the die-off of very large numbers of L. stagnalis which littered the sedge mats and shoreline with decaying snail bodies and shells. *This usually occurred rapidly with no dead snails being* observed two weeks previously during early September. With the onset of colder temperatures, egg production slowed dramatically. Several adult snails collected in the middle of October were brought into the lab but produced no eggs for one week, and then produced only small egg masses for another five days. After 12 days larger, more normal appearing egg masses were produced. Those adults which overwintered

and were brought into the laboratory immediately produced relatively normal egg masses. Apparently the onset of progressively colder temperatures conditioned the molluscs to a slow-down and eventual cessation of gamete production, which required an appropriate start-up time to re-initiate egg laying. In the spring when there is a progressive warming of the habitat, there appears to be an activation of gametogenesis.

Further analysis of the snail growth data was performed by graphing sample means and standard deviations, and fitting this information into an equation which accurately described growth. The growth equation by von Bertalanffy (1938) had been used most commonly to describe growth in molluscs. This equation, however, did not clearly demonstrate changes occurring during early growth in snails.

Plorin (1984) has shown that the Logistic Equation developed by Robertson (1923) and usually used to describe growth in fish, accurately predicted growth occurring in the shorter-lived molluscs, such as planorbid and lymnaeid snails. According to Plorin, parameter estimates for the Logistic Equation were calculated using a modification of the Method of Finite Differences (Rasor, 1949). These parameters were dependent on measurements being taken at equal time intervals. A good fit was not possible if the data differed significantly from the ideal curve.

Results of fitting the growth data for 1976 and 1977 to the Logistic Equation are seen in Fig. 4. Calculations for this process may be found in the appendix, Table A-2. A good fit was possible for both years, and demonstrated more clearly the variation seen during

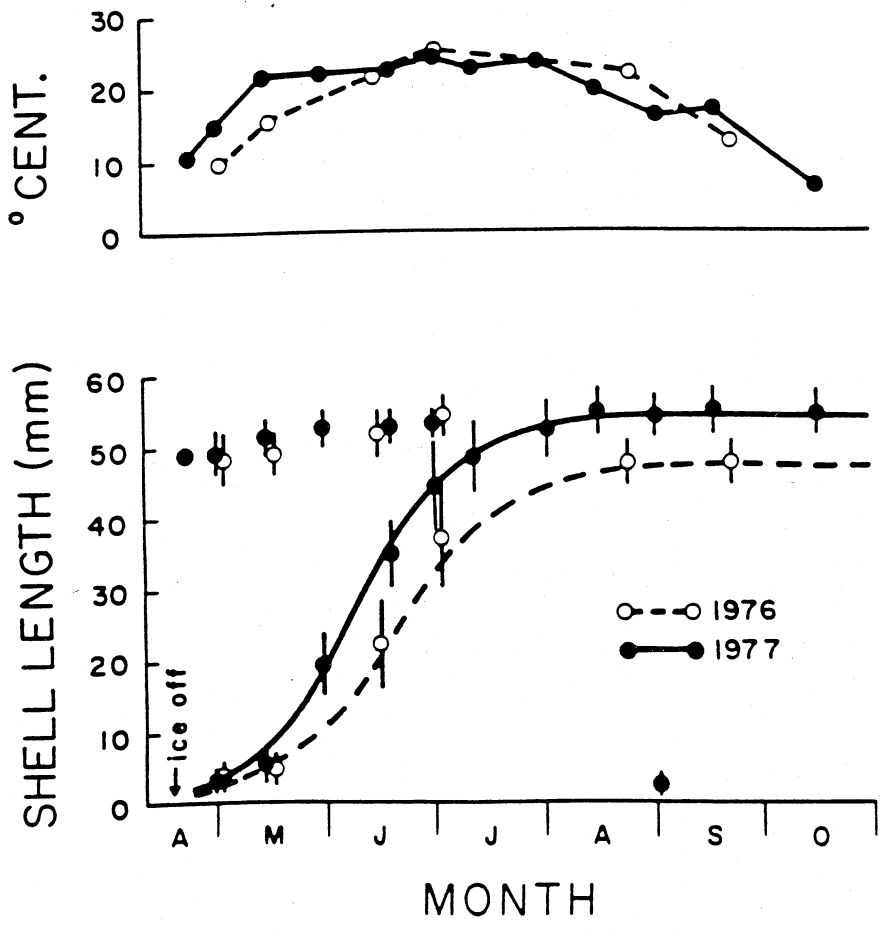

Figure 4. Growth curves* of 1543 L. stagnalis appressa for 1976 and 1977 from data fitted to the Logistic Equation (Robertson, 1923).

*Each point represents a sample mean ± 1 standard deviation of total shell length of snails collected from April through October in Nicollet Lake. Lake temperatures are indicated at a depth of approximately 10 cm.

that time. After maxmimum growth was attained by mid-summer, the sample mean for 1976 was 47 mm whereas that for 1977 was 54 mm. The growth curve for 1976 showed a retardation of approximately 2 weeks relative to 1977.

This variation may be explained by examining differences seen in the water temperature profiles (Fig. 4). During 1976 equivalent water temperatures lagged almost 2 weeks behind those of 1977 (16°C on 5-15-76 and 15°C on 4-30-77, climbing to 22°C on 5-14-77). A further demonstration of this phenomenon may be seen in Fig. 5, in which growth rates for both years have been generated from information provided by the Logistic Equation. Peak growth rates occurred on or about June 23, 1976 and on June 11, 1977, resulting in a 12-day retardation of the former relative to the latter. This information closely agreed with the variation in ambient water temperatures seen for those years. Such differences in environmental conditions have been shown to result in considerable infraspecific interpopulation variation in growth rates and reproduction for species such as Gyraulus albus (Russell Hunter, 1961, 1964), Physa gyrina (DeWitt, 1955), L. humilis (McCraw, 1961), L. stagnalis (L.) (Berrie, 1965), and others.

Baxter (1973) addressed the role that overwintered adults played in the population dynamics of L. stagnalis. Her studies indicated that these snails had a low rate of reproduction in the spring and did not survive much beyond mid-July. They had, therefore, little effect on the population. The present study essentially agreed with these findings and provided some additional details.

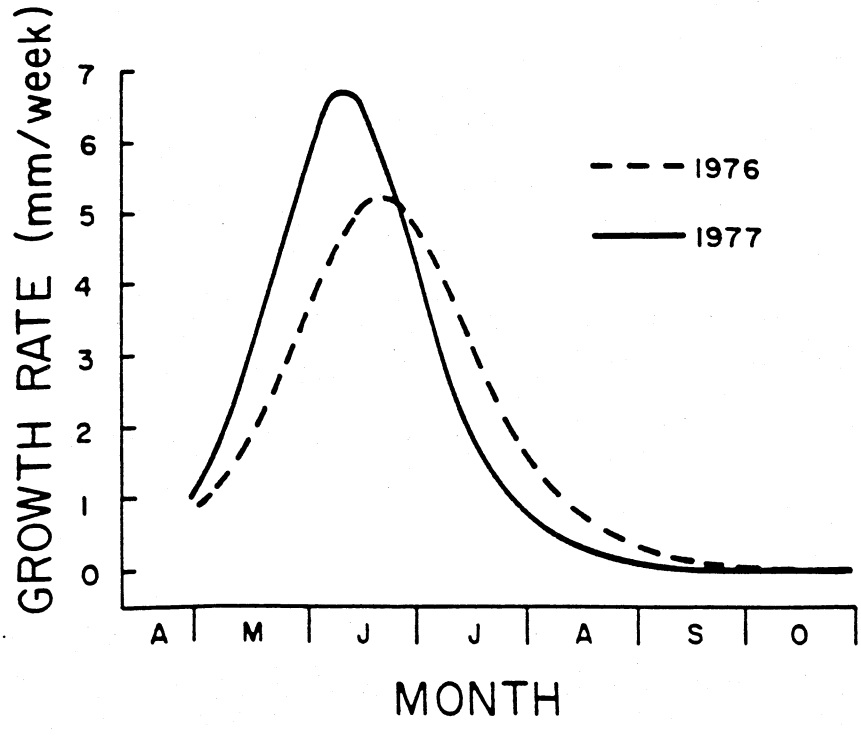

Figure 5. Growth rates of L. stagnalis appressa for 1976 and 1977 from data fitted to the Logistic Equation (Robertson, 1923) (see Table A-2)*.

*Peak growth occured on or about June 23, 1976 and June 11, 1977.

The egg masses laid by overwintered snails in Nicollet Lake appeared somewhat smaller than normal, but enough were present to be easily found throughout the spring. Careful examination of the altitude-frequency histograms for both years demonstrated that newly hatched spring snails were present in the samples as "stragglers' during June and July, and resulted in a skewing of the distributions to the left. These were differentiated from the overwintered young snails (2-5 mm) which grew at a more uniform rate during this time. The spring hatched snails were also partly responsible for the markedly increased sample standard deviations seen during this time (standard deviations ranged from 1 to 3 mm during most of the year, but increased to greater than 6 mm during June and July). These individuals made up 3-5% of the younger crop which could have proven to be a significant number should excessive mortality have occurred in juvenile snails during the fall or winter.

Development of L. stagnalis from laying of the egg mass to egg-laying adult required a minimum of two and one-half months under peak summer conditions (van der Schalie and Berry, 1973). This time period is adequate for spring-hatched snails to catch up with and be incorporated into the primary population, with egg laying then occurring by the middle of summer. The overwintering of both adult and juvenile snails certainly provides added protection to the species from catastrophic environmental change for which one or the other group may be particularly susceptible. Population changes in such a circumstance would then be compensated for by modified fecundity, often within one reproductive period (Eisenberg, 1966; Baxter, 1973).

Lymnaea stagnalis has been considered to be one of the most characteristic as well as the largest of the lymnaeids, its presence and success dependent on numerous environmental factors, many of which were discussed earlier. As Boycott (1936) found, lymnaeids required substantial amounts of calcium in their environment. This would seem to be especially true where considerable growth occurs over a short summer period. Nicollet Lake provided such a habitat with high Ca^{++} and total alkalinity (see Table 4), that resulted from the surrounding calcareous type of grey glacial drift. The high fertility seen in this lake was reflected in the increased growth seen in L. stagnalis. Many papers have mentioned the size (total height) of specimens of L. stagnalis collected (see Table 7). The large sizes routinely found in Nicollet Lake were comparable to some of these reports. The largest specimens recovered were 63.7 mm, found on 9-17-77 and 9-24-79. Such large specimens were unusual, even for the lakes and streams surrounding Nicollet Lake. The possibility of parasitism by larval trematodes causing such unusual growth has been discussed elsewhere in this paper.

The variability seen in the literature regarding the normal life span of various pulmonates (Table 1) reflects both infraspecific and interspecific differences. According to Berrie (1965) egg-laying in L. stagnalis (L.) in Scotland started towards the end of May and peaked in mid-June. This also appeared to be true for the other species of lymnaeids discussed previously. Most of the reproductive activity in L. stagnalis appressa however, has been reported to take place from mid-summer to early fall (Baker, 1911; Baxter, 1973;

Table 7. References to either individual or averaged* recorded lengths of *L. stagnalis*

Reference	Species	Total Length (mm)
Say (1818)	*L. stagnalis appressa*	42
Baker (1911)	" " "	57.5
Crabb (1929)	" " "	44 (lab raised)
Cheatum (1934)	" " "	45*
Noland and Carriker (1946)	" " "	62.5 (lab raised)
Clarke (1973)	" " "	59.7
Baxter (1977)	" " "	54*
Present Study	" " "	63.7
Hogg (1854)	*L. stagnalis* (L.)	47
Baker (1911)	" " "	52
Wesenberg-Lund (1934)	" " "	50
Berrie (1965)	" " "	35

McDonald, 1969; Noland and Carriker, 1946).

The life cycle of this species in Nicollet Lake is primarily that of an annual. Most egg laying occurs in late July and August with individuals from this hatch surviving until the middle of September the following year (13-14 months) after which most die. Small but significant numbers may overwinter a second time and reproduce the following spring. These second year snails do not appear to survive much beyond early July, but this biennial capability does extend the life span to a maximum of 23 to 24 months. The importance of their reproductive potential in population regulation is unknown, but may be of significance in situations where unusual fall and winter conditions result in excessive mortality of young snails.

Berrie (1965) suggested that the biennial life cycle of L. stagnalis (L.) was an adaptation to marginal habitat conditions, but this would not seem to be the case in Nicollet Lake. Rather, this phenomenon may be more common, and simply the result of individual longevity. Possibly, these individuals may have been the progeny of other overwintered adults, hence were hatched in spring and overwintered only as adults, never as juvenile snails. This, of course, implies that their life span is no greater than that of the late summer hatch (13-14 months).

Finally, the life span for some snails may be as short as 4-5 months. Those which hatch in mid-to-late May from overwintered adults will reach their reproductive age by late July, and probably die in mid-September along with the annuals.

While most L. stagnalis in Nicollet Lake have an annual life

cycle and overwinter once, some may extend this up to two years and two breeding seasons by overwintering twice. Others may conceivably hatch, reproduce, and die in less than half a year. This variation may be of significance in protecting the population from adverse environmental conditions.

ECOLOGICAL RELATIONSHIPS OF *COTYLURUS FLABELLIFORMIS*

Seasonal infection rates of *L. stagnalis* collected in Nicollet Lake from 1976 to 1979 for commonly found cercarial types have been listed in the appendix (Table A-3). Cercariae of *C. flabelliformis* and an unidentified xiphidiocercariae were by far the most plentiful. *Trichobilharzia ocellata* (*Cercaria elvae*) was found infrequently from April to July. Only one double infection was found and contained sporocysts of *C. flabelliformis*, and those of an unidentified cercaria with lateral and dorso-ventral finfolds, possibly of the parapleurolophocercous type. Calculation of the expected frequency of double infection (product of the infection rate times the number of snails in the collection)(Bourns, 1963) for each collection was always less than one, which was not surprising given the generally low incidences of sporocyst infection present in Nicollet Lake. Conclusions regarding the effect that prior infection had on future susceptibility to infection could not be ascertained from this information, except that it did not appear to be increased.

Seasonal changes occurring in the percentage of *L. stagnalis* shedding *C. flabelliformis* cercariae showed significant yearly fluctuations (Fig. 6). Snails which hatched in the summer and fall of 1975 and overwintered as juveniles first began shedding cercariae the following June, 1976. Most of the later summer infections originated during the spring migration, but some (especially those becoming patent in mid-June) may have been acquired the previous fall, remaining undeveloped during the winter. As summer progressed this number increased to 18%. This was very similar to the number

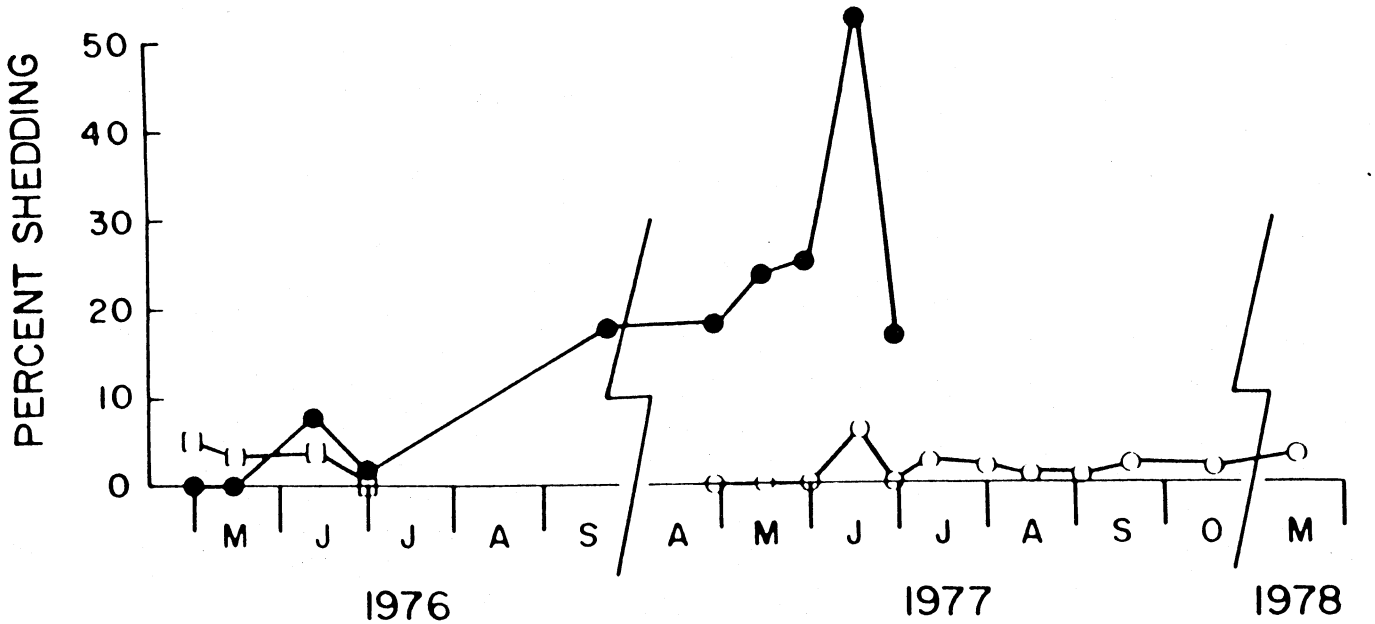

Figure 6. Percent* of L. stagnalis appressa shedding C. flabelliformis cercariae during the years 1976 to 1978.

*Two distinct age classes are evident in Spring of 1976 and 1977, with percentages of both being represented.

recovered the following spring, and indicated that the same proportion of infected to non-infected snails survived the winter intact without differential mortality. As the spring of 1977 progressed however, the percentage of infected snails increased steadily until the end of June when the percentage declined, followed by the disappearance of these snails from the collections. Although the numbers of overintered specimens were small, this pattern suggests that the sporocyst-infected snails live longer than uninfected snails. This has been shown to be true for L. stagnalis infected with T. ocellata (Bourns, 1969). The other possibility is that infections acquired the previous fall (possibly during the fall migration) were becoming patent as spring progressed. This is doubtful however, considering that the sporocyst-infected snails contained few tetracotyles (mean of 45 per snail on 6-18-77), implying exposure to miracidia when the snails were quite young.

Those snails which hatched in the summer and fall of 1976 and overwintered as juveniles, first developed patent infections in June, 1977 (same as in 1976). As this group progressed through the summer incidence of cercarial shedding remained at 1 to 3%, and did not increase in the fall as had occurred the previous year. A collection made the following spring (May, 1978) corroborated the fall value. Similarly, overwintered adults appearing in the spring of 1976 show a low incidence of infection.

Reasons for this year to year variation remain unclear. Considering that the life cycle studies of L. stagnalis showed similar changes for both years, changes occurring in the habits of final hosts

must be suspect. The presence or absence of nesting waterfowl in addition to the spring and fall transients may be an important limiting factor of these parasites in a lake as small as Nicollet (7.2 acres). Similar variation was also noted to occur with the incidence of xiphidiocercariae in L. stagnalis, except in opposite years. The prevalence of this parasite remained low (2 to 6%) during 1976 and the first half of 1977, whereupon it increased to 20% in the fall of that year and spring of the next (May, 1978). Although it is tempting to speculate as to the influence one parasite may have on another, no conclusion can be drawn in this circumstance. Interestingly, Bourns (1963) documented that xiphidiocercariae and C. flabelliformis occurred together as multiple infections far more frequently than would be expected by chance. Although both of these species occurred commonly in L. stagnalis in Nicollet Lake, no multiple infection with this combination was found.

The seasonal changes occurring in snails infected with C. flabelliformis sporocysts differed somewhat from those seen by Campbell (1973b) in Iowa. In those studies the peak of cercarial shedding (35-60%) varied from August to September, with the percentage declining significantly thereafter. A small peak in the spring resulted from the overwintering of infected adult snails. In the present study the percentages of snails shedding cercariae in the fall matched that seen in the spring, although year to year variation was quite marked. During 1977 (Fig. 6), there was no peak in cercarial shedding--just persistantly low numbers throughout the summer and fall, and into the spring of 1978.

Tetracotyle metacercariae of C. flabelliformis were commonly encountered in a variety of molluscs and, when present, often occurred in large numbers (Bourns, 1963; Cort, Brackett, and Olivier, 1944; Nolf and Cort, 1933). By definition, the metacercaria that reaches the tetracotyle or encysted stage is infective to the definitive host. During the summer in Iowa, Ulmer (1955, 1957) found that 99% of L. reflexa harbored developing and fully formed tetracotyles of this species. L. stagnalis appressa from Nicollet Lake were likewise heavily infected with Tetracotyle flabelliformis (one specimen contained 4,030). All snails of this species greater than 31 mm in shell altitude contained developing and mature tetracotyles. The smallest snail harboring a tetracotyle was 18 mm, which was undoubtedly close to the lower size limit where one might expect tetracotyles to be found. The time required for a snail to achieve this size is about 40 days, similar to that required for metacercarial development at ambient water temperatures (42+ days at 12-14°C; 18-24 days at 22-24°C, according to Campbell, 1973c). Developing forms of metacercariae were found in some of the smallest snails examined (12 mm) and were probably present in much younger specimens. In the laboratory Cercaria flabelliformis readily penetrated and greatly irritated young snails only recently hatched.

Counts of the numbers of tetracotyles found in individual snails were made periodically from 1976 to 1979. Because each collection contained only one or, at the most, two size classes of snails, results of counts from a number of collections were pooled to provide a continuous spectrum of shell length. Once tetracotyles were found

in younger snails, their numbers increased exponentially with shell size. These changes were best visualized by plotting the log number of tetracotyles versus shell length (Fig. 7). Each point marked on the figure represents the log number of tetracotyles found in the dissection of an individual snail. Those snails without tetracotyles were not included in this graph, as the required log (X+1) transformation would unduly weight the lower end of the scale, preventing accurate trend analysis. The method of least squares linear regression was then applied to this data resulting in the "best fit" line with a correlation coefficient of 0.914 (highly significant).

This regression line suggested that the number of tetracotyles per snail was limited more by the size of the individual than to the density of cercariae in the environment, once above a certain threshold level. Cort, Brackett, and Olivier (1944) and others demonstrated also that metacercariae display a marked retardation in development in heavily infected snails, probably resulting from the competition for available nutrients and space. Campbell (1972) added that the immature condition of the ovotestis with fewer follicles in young snails may explain the smaller number of metacercariae found. Both studies implied that the intensity of parasitization was dependent on host size. Considering that small numbers of snails were shedding large numbers of C. flabelliformis cercariae from spring to fall (Fig. 6), all snails were constantly exposed and would be expected to accumulate tetracotyles at a variable rate proportional to, but limited by, their size.

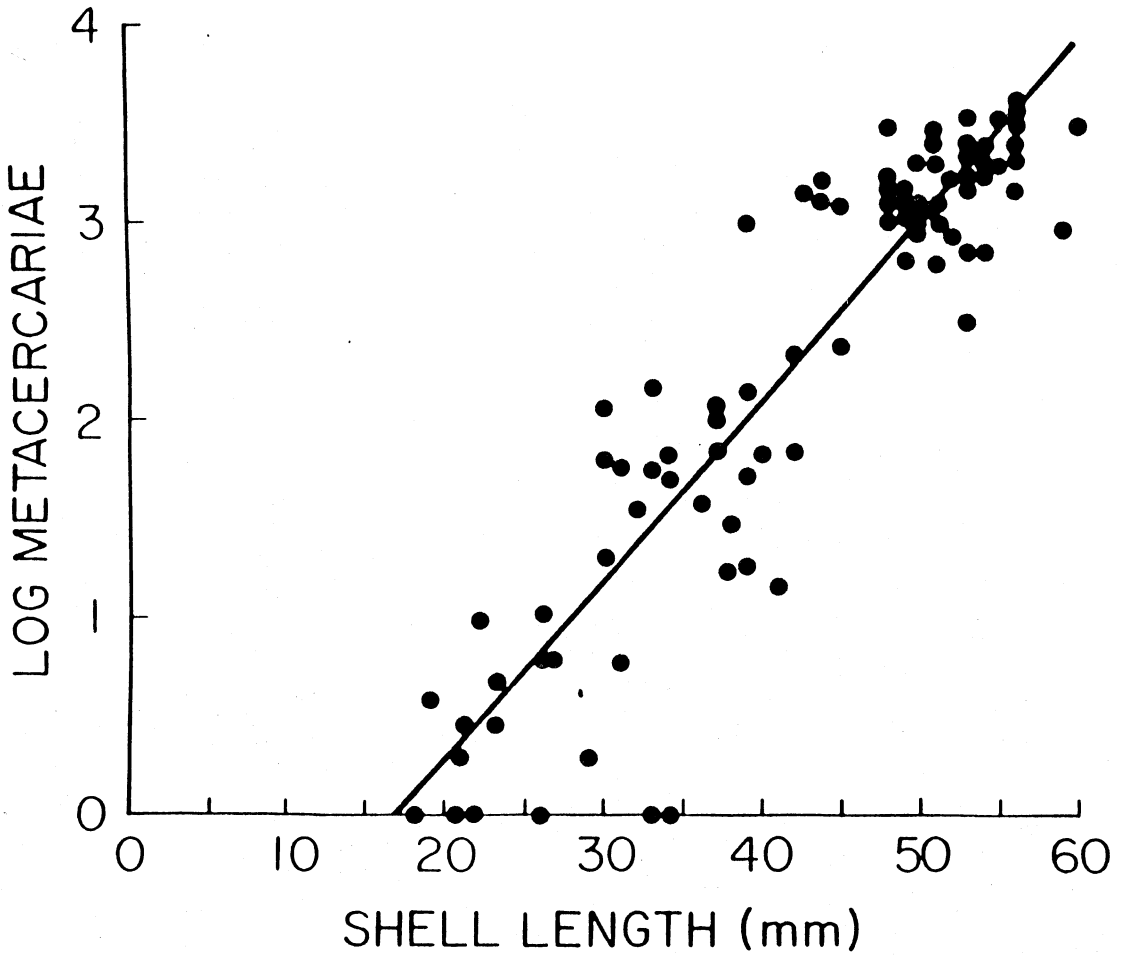

Figure 7. Log number of tetracotyle metacercariae found in individual L. stagnalis compared to shell length*, from snails collected periodically during the years of 1976 to 1979.

*The regression line was constructed using the method of Least Squares. Those snails without tetracotyles are not included.

The high correlation seen with this data makes the regression a useful predictor of changes occurring in the parasite population as snail growth (and hence age) proceeds through the year. Changes occurring in shell length over time were previously described (Tables A-1, A-2 and Fig. 4). Numbers of tetracotyles were easily calculated from the regression analysis for any date by using snail size as predicted by the Logistic Equation (calculated values are found in appendix Table A-4). The result of this process was Fig. 8, which depicts the seasonal changes (1977) occurring in the total number and log number of tetracotyles per snail for those individuals overwintering in the 2 to 5 mm size classes. The calculated values of the log curve were compared for accuracy with direct counts made on selected dates (appendix Table A-5), and showed a reasonably good fit. The log curve provides a better visualization of changes occurring in the accumulation of tetracotyles early in the season. From this graph we see that tetrocotyles first start appearing in the young snails during the end of May-beginning of June. Considering the warm water temperatures for most of May that year (Fig. 4), cercariae released from overwintered adults in the beginning of the month would have had sufficient time for development to the tetracotyle stage. Undoubtedly, some were also acquired the previous fall, but remained undeveloped or only partly developed because of cold temperatures. The thousand-fold increase in the number of tetracotyles that appeared in snails occurred during July. Thereafter, the numbers leveled off, paralleling the static period of snail growth from August onward.

The greatest increase in total number of tetrocotyles however,

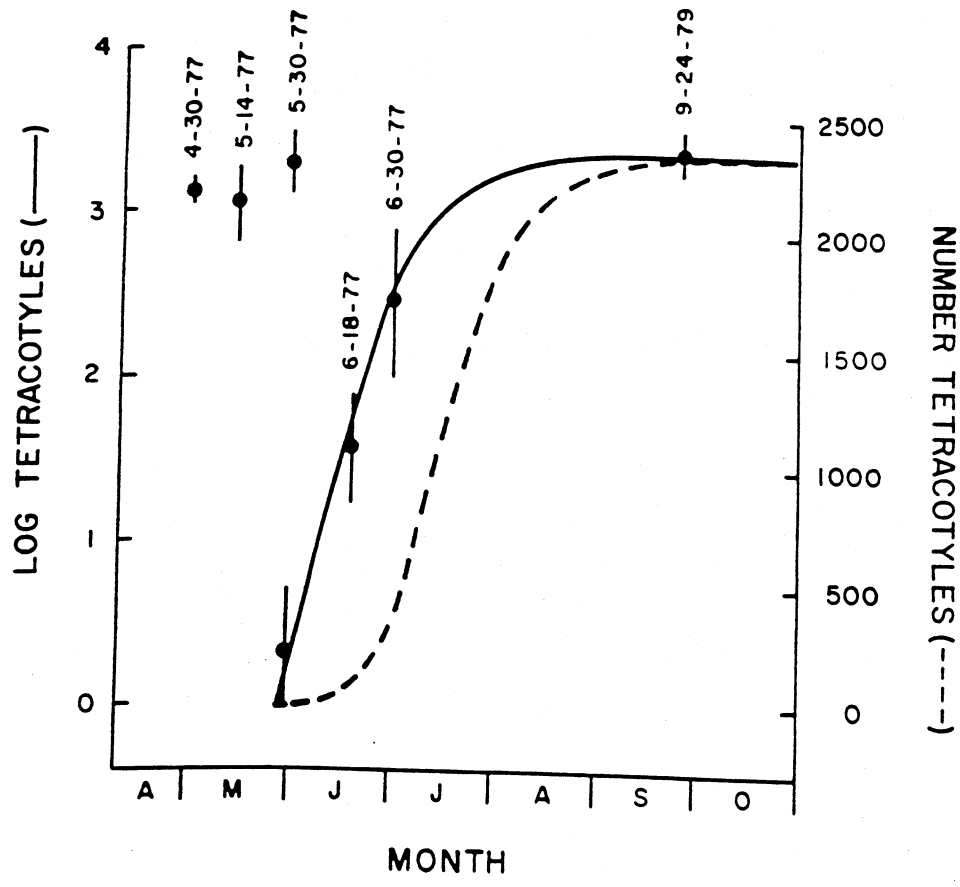

Figure 8. Seasonal changes occuring in the total number and log number of tetracotyles per snail*.

*Numbers of tetracotyles are calculated from the regression analysis for any date by using snail size as predicted by the Logistic Equation (see Table A-4). Direct counts made on collections from selected dates are included for comparison (see Table A-5).

occurred shortly before the snails reached maximum size. By comparing the changes in percentage of total tetracotyles per snail with the percentage of maximum achievable shell size (Fig. 9 and appendix Table A-4) it was seen that snails completed over 50% of their growth before even 1% of their tetracotyles appeared. Those snails reaching 80% of their final size contained only about 10% of their total tetracotyles. The importance of these large snails in providing infective material to the final host is uncertain. Campbell (1973b) recovered only smaller snails (up to 35 mm) from the stomachs of wild ducks, but found that tetracotyles contained in the bodies of floating dead snails remained infective. The large numbers of dead snails (without shells) seen floating in Nicollet Lake during the spring and fall should provide a ready source of infective material even if living snails of this size do not.

This same information was used to calculate the rate of change in numbers of tetracotyles that appeared in snails throughout the year (appendix Table A-4). When this was compared to the growth rate of L. stagnalis for 1977 (Fig. 10), the peak of infection (greatest number of tetracotyles acquired in one week) occurred 34 days after peak growth. The developmental times required for tetracotyle formation at July water temperatures (20 to 24°C) should be slightly less than this (by about 10 days). The extra time required for encystment might have been the result of the presence of large numbers of metacercariae, resulting in retardation of their development as described previously by Cort, Brackett, and Olivier (1944).

Taking into account the time delay for metacercarial encystment,

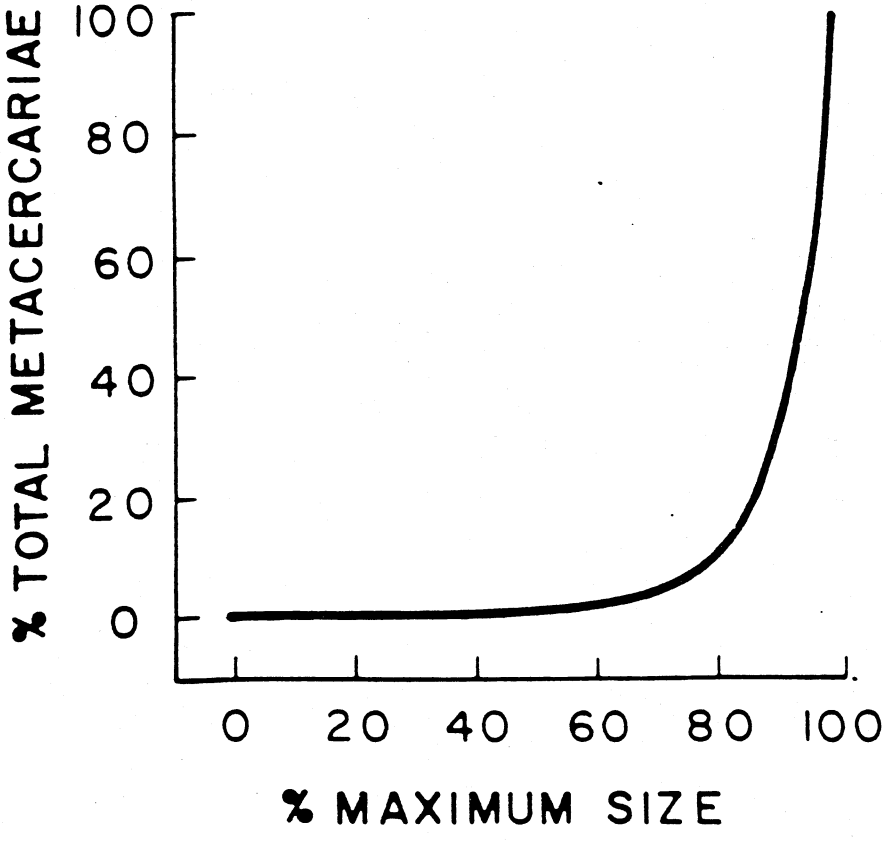

Figure 9. Changes occuring in the percentage of total tetracotyles per snail as the host achieves maximum shell size (see TABLE A-4).

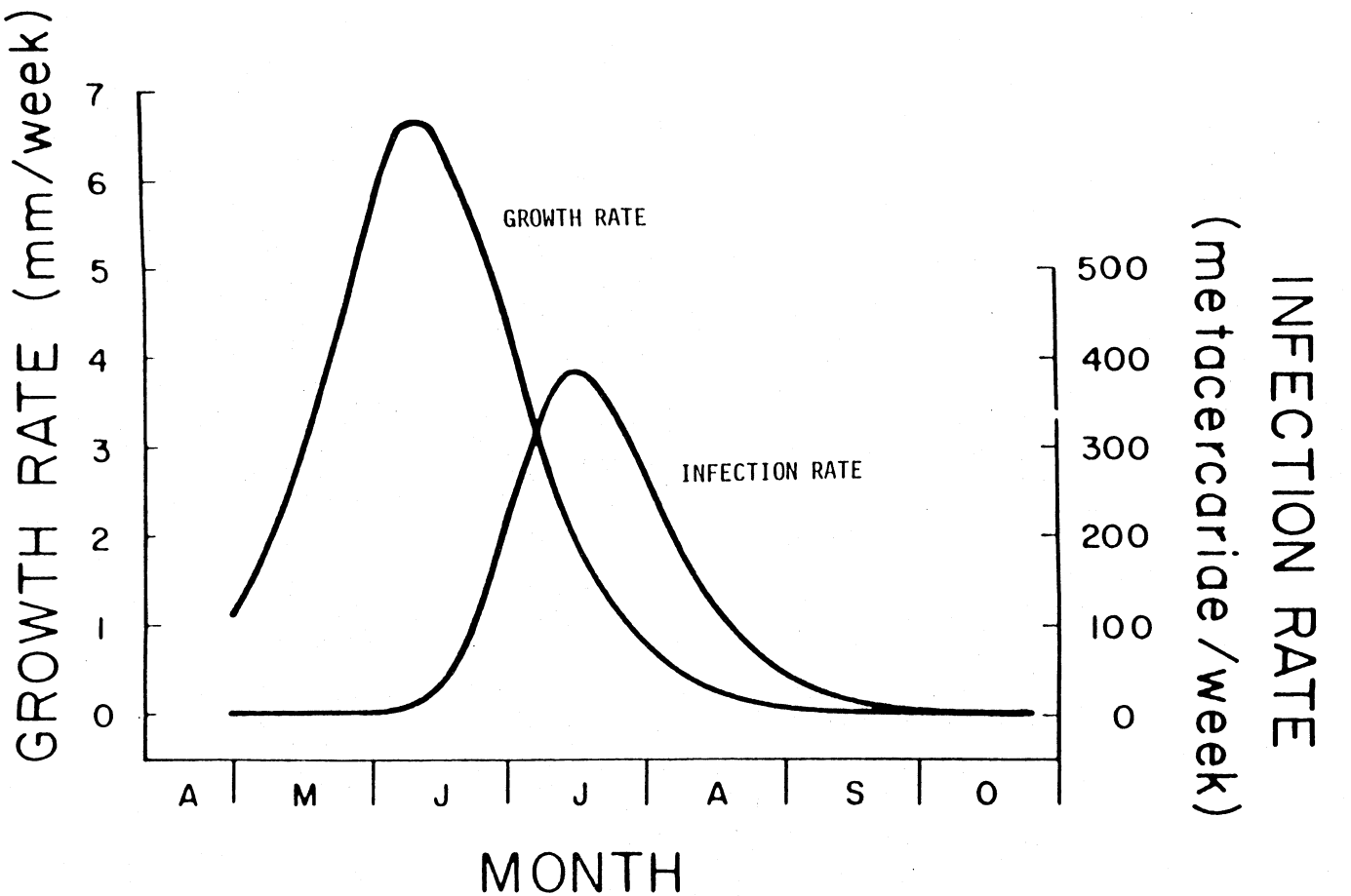

Figure 10. The infection rate of L. stagnalis with tetracotyle metacercariae is displayed alongside the growth rate of the mollusc itself for 1977*.

*The offset peaks are partially explainable by the developmental times required for tetracotyle formation at summer temperatures.

the peak cercarial penetration rate occurs almost simultaneously with the peak snail growth rate (around June 11 in 1977). Few snails of that age would have had time to develop patent sporocyst infections and produce cercariae in large enough numbers to account for this peak. Those large overwintered adult snails which are shedding C. flabelliformis cercariae, however, would be able to fulfill this role, and to persist in the population through the end of June. By that time small numbers (1-6%) of younger snails are also shedding cercariae, maintaining exposure to all snails. In addition to providing a source of infective tetracotyles to migrating waterfowl in the spring, overwintered adults also provide cercariae from which a large percentage of tetracotyles in younger snails will ultimately develop.

While the pattern of seasonal changes in the appearance of tetracotyles in Nicollet Lake was similar to that recorded by Campbell (1973), significant variation does exist in some of the details. As in Iowa a bimodal increase was seen with the largest numbers occurring in the spring (in overwintered adult specimens) and fall. In the present study however, over one-half of the total number of tetracotyles per snail appeared by mid-July, whereas in Iowa the corresponding increase did not occur until mid-September. Intensity of infection varied likewise with a peak average of 2,300 tetracotyles per snail in Nicollet Lake compared to 700 per snail in Iowa (1,000 or more in larger individuals). Most certainly these differences reflect those seen in the intensity of sporocyst infestation between the two habitats. In Nicollet Lake the high proportion of overwintered snails

shedding cercarae in the spring of 1977 (Fig. 6) were most likely responsible for the early summer appearance of large numbers of tetracotyles. The low level of metacercarial infections seen in mid-summer in Iowa may have reflected the low incidence of sporocyst infestation in overwintered snails.

Seasonal changes in C. flabelliformis infections (both sporocyst and metacercarial) of L. stagnalis in Nicollet Lake from April to July, 1977, are summarized in Fig. 11. Cercariae released from overwintered sporocyst-infested snails were first noted as developing forms in younger snails on May 30. All snails contained tetracotyles after June 30. Tetracotyles found in the overwintered adult snails provided infective material for migratory waterfowl, and resulted in the appearance of patent sporocyst infections in younger snails by June 18. Sporocyst-infested snails comprised an increasing percentage of overwintered adults throughout the spring. The role overwintering adults play in maintenance of the snail population may be limited, but their presence is of vital importance in maintaining a stable parasite population.

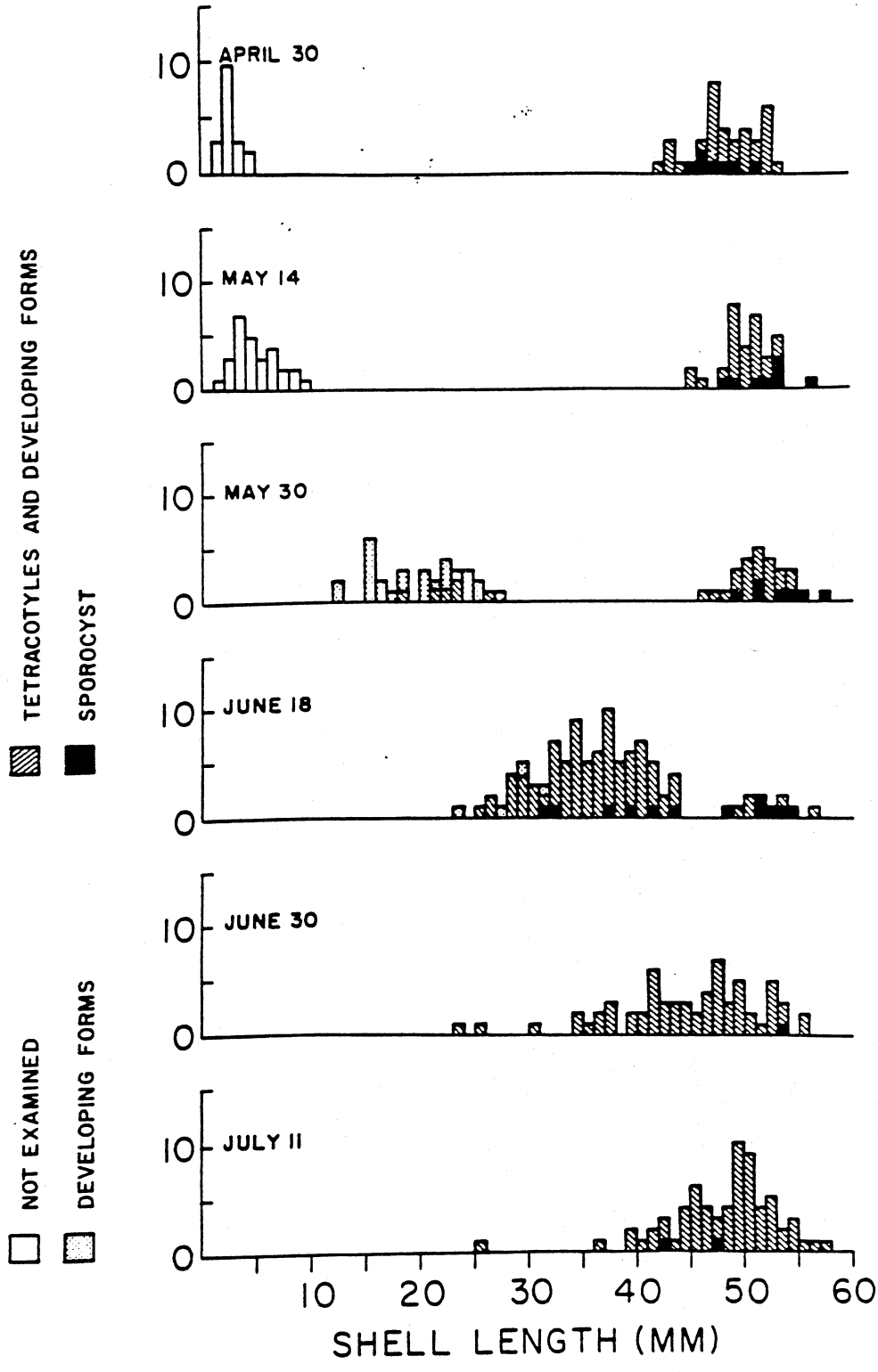

Figure 11. Seasonal changes in Cotylurus flabelliformis infection of Lymnaea stagnalis in Nicollet Lake, Clearwater County, Minnesota, from April to July, 1977.

INTRASPECIFIC AND INTERSPECIFIC TREMATODE ANTAGONISM IN THE SNAIL HOST

Antagonistic interactions between larval trematodes occur in a variety of situations. The "Winfield Effect" as coined by Basch (1970) referred specifically to the inability of cercariae of certain Cotylurus species to develop into tetracotyles in snails already infected with sporocysts of the same species. The paper by Cort, Brackett, Olivier and Nolf (1945) summarized the research on this topic with C. flabelliformis.

The present study expanded on this information by comparing counts of tetracotyles found in sporocyst-infected and uninfected snails, mindful of the size and age of the snail host. Results are presented in Fig. 12 with the data fitted by Least Squares Linear Regression analysis. Numbers of tetracotyles in sporocyst-free snails displayed good linearity with a correlation coefficient of 0.914, whereas those containing C. flabelliformis sporocysts showed wide variation in numbers of tetracotyles, and a low (essentially non-linear) correlation coefficient (0.101). Comparison of the correlation coefficients by the Fisher Method (Fisher, 1921) did, however, demonstrate a significant difference ($p<.01$) between the two, supporting the visually obvious disparity between sporocyst-infected and sporocyst-free snails.

We see from this graph that most sporocyst-infected snails harbored less than 100 tetracotyles each, and many of these occurred as hyperparasites within daughter sporocysts. Some however, did contain large numbers of tetracotyles (up to 1,000), equivalent to

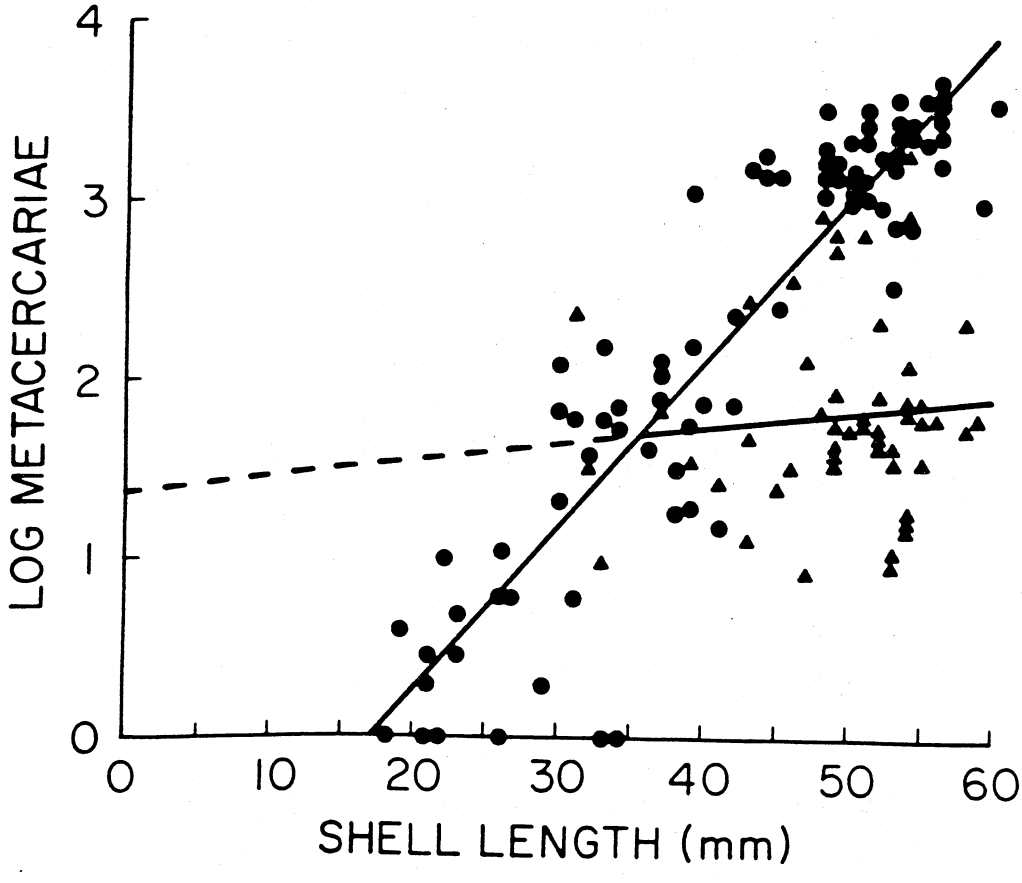

Figure 12. Counts of tetracotyle metacercariae found in sporocyst-infected (▲) and sporocyst-free (●) L. stagnalis compared to shell length*.

*The data were fitted by Least Squares Linear Regression, and the correlation coefficients found to be significantly different (p .01).

sporocyst-free snails. Winfield (1932) speculated that these few individuals were exposed to miracidia after large numbers of cercariae had already penetrated and started their own development. Reasons for finding such small numbers of doubly infected snails may include 1) increased host mortality to double infection, or 2) the refractory characteristic older snails often exhibit to miracidial penetration and sporocyst development.

All snails shedding C. flabelliformis cercariae (the youngest being 31 mm) contained some tetracotyles. Likewise, all sporocyst-free snails of greater than 31 mm also contained tetracotyles. The small number of tetracotyles found in most sporocyst-infested snails implies exposure to some cercariae when the snail is quite young, probably before miracidial exposure. After miracidial penetration and early development of the sporocyst, cercariae do not penetrate in large numbers and/or do not develop. The limiting factor appears to be the size of the sporocyst mass and whatever product it may produce that inhibits cercarial penetration and metacercarial development. If some inhibitory product were not present, we might expect large numbers of cercariae to spontaneously undergo metamorphosis without ever leaving the snail. Basch (1970) has conclusively shown, with C. lutzi, that this inhibition was present only as long as the sporocysts were present. This same mechanism appears to be working with C. flabelliformis.

Antagonistic interaction between C. flabelliformis and another trematode species, S. douthitti, was first described by Nolf and Cort (1933). These authors observed that significantly smaller numbers of

C. flabelliformis metacercariae were present in S. douthitti-infected L. stagnalis than in control groups. To examine this phenomenon further, uninfected and S. douthitti-infected L. elodes were exposed to similar numbers of C. flabelliformis cercariae. Results from these experiments (Table 8) confirmed the inhibitory effect that schistosome sporocysts have on the development of C. flabelliformis metacercariae. Significantly larger numbers of tetracotyles developed in uninfected L. elodes than in S. douthitti-infected snails. In addition to the difference in total numbers of metacercariae recovered, rate of development was also different. All metacercariae had reached the tetracotyle stage after 20 days in uninfected snails, whereas slightly less than half the metacercariae in schistosome-infected snails were still present as developing forms. The presence of schistosome sporocyst in L. elodes (as in L. stagnalis) did affect both the number of tetracotyles that ultimately appeared and the rate at which they developed.

The presence of xiphidiocercariae-producing sporocysts in field collected L. stagnalis was also associated with a partial aversion to the establishment of tetracotyle metacercariae. This aversion was inconstant, with numbers of tetracotyles varying widely (see Table below).

In some instances the aversion was equal to that seen in C. flabelliformis sporocyst-infected snails, and in others no aversion was noted. These findings in L. stagnalis are similar to what Cort, Brackett, Olivier, and Nolf (1945) found in Stagnicola emarginata angulata containing stylet cercariae of the family Plagiorchiidae. In

TABLE 8. Metacercariae of C. flabelliformis recovered 20 days post-infection from Lymnea elodes exposed to 50 cercariae per snail, with and without prior exposure to S. douthitti.

		MEAN ± SEM (RANGE)	
	No. of Snails	Developing Forms	Tetracotyles
L. elodes uninfected	11	0	12.2±2.2 (4-29)
S. douthitti-infected	15	2.1±0.7 (0-8)	3.7±0.6 (0-7)
p value		$p < 0.025$	$p < 0.001$

that study snails infected with P. proximus contained numbers of metacercariae comparable to that of uninfected snails, whereas P. muris-infected snails contained only small numbers of metacercariae, similar to those infected with C. flabelliformis sporocysts.

XIPHIDIOCERCARIAL-INFECTED L. stagnalis

SHELL LENGTH (mm)	TETRACOTYLES RECOVERED
35	210
42	69
43	90, 230
44	15
47	430
48	120, 840
50	109, 730
51	2473
52	55, 880, 1680
53	130
54	1700
55	250, 2180
57	900

Other experiments by Cort, Brackett, and Olivier (1941) found that C. flabelliformis cercariae actively penetrated planorbid and physid snails, but that no metacercarial development occurred unless sporocyst or redial stages of certain other trematodes were present. In the latter case the metacercariae usually developed within the germinal sacs of the sporocyst as hyperparasites. Non-host snails were utilized to further examine these unusual host specificity patterns. Specifically, uninfected and S. mansoni-infected B. glabrata (albino strain) were exposed to C. flabelliformis cercariae, and examined after a time interval sufficient for metacercarial development. Uninfected Helisoma trivolvis exposed to similar numbers of cercariae were used for comparison.

Results (Table 9) indicated that numerous tetracotyles developed

TABLE 9. Metacercariae of C. flabelliformis recovered 18 days post-infection from Biomphalaria glabrata and Helisoma trivolvis exposed to 100 cercariae per snail. One group of B. glabrata had been previously infected with S. mansoni.

		MEAN ± SEM (RANGE)	
	No. of Snails	Developing Forms	Tetracotyles
B. galbrata uninfected	15	41.9±3.5 (28-65)	2.9±1.1 (0-12)
S. mansoni-infected	5	17.4±3.8 (9-29)	44.5±8.0 (20-63)
p value		$p < 0.005$	$p < 0.001$
H. trivolvis uninfected	15	0	0

in S. mansoni-infected snails, as predicted by Cort's findings. In contradiction to those findings, however, numerous large developing forms as well as several normal appearing tetracotyles were found in sporocyst-free B. glabrata. No metacercariae were found in any of the H. trivolvis, although cercariae were seen to actively penetrate all snails and degenerating cercarial bodies were present in the tissues of the foot. The susceptibility of B. glabrata to infection with this strigeid was greater than that of the other planorbid. Reasons for this discrepancy were unclear, but may have resulted from the fact that distributions of these two organisms do not overlap in nature, preventing the establishment of a coevolutionary response.

Except for the work of Basch (1970), little information has been added to the understanding of the "Winfield Effect" in C. flabelliformis since the work at Douglas Lake by Cort et al in the 1930's and 1940's. The present study has re-examined certain details of this phenomenon as it applies to the second intermediate host relationships involving lymnaeid and planorbid snails. Generally speaking, the presence of sporocysts (especially parasite mass in relation to total host mass) appears to be a limiting factor for tetracotyle development in lymnaeids, and a required factor for tetracotyle survival in planorbids. The two mechanisms responsible are most likely quite different. In lymnaeids the type of parasite inhibition seen suggests that cercarial penetration and metacercarial metamorphosis is slowed or prevented by some substance elaborated by the sporocyst. This would prevent over-parasitization, undue competition for host nutrients, and possibly host death. In

planorbids however, the sporocyst appears to offer a protected environment, one in which metacercariae rapidly develop as hyperparasites outside the influence of an otherwise hostile environment.

PATHOLOGICAL EFFECTS OF C. FLABELLIFORMIS ON HOST AND NON-HOST MOLLUSCA

Effects on Snail Growth:

Larval forms of a number of trematode species have been known to either enhance or inhibit growth characteristics, reproduction, and/or longevity of their appropriate molluscan hosts. McClelland and Bourns (1959) found that growth of L. stagnalis was enhanced by infection with T. ocellata, whereas Zischke (1967) found the opposite to be true for Stagnicola palustris infected with E. revolutum. Pathogenesis in molluscs associated with trematode infection includes both the direct and indirect effects that parasites have on the host's digestive and reproductive organs.

The effects that infection with Cotylurus flabelliformis have on growth, reproduction, and longevity of L. stagnalis are unknown. The sporocyst stage develops primarily within the hepatopancreas (digestive gland) of the snail, whereas the tetracotyles tend to accumulate within the ovotestis (hermaphroditic gland). This situation should provide the opportunity for stunting by nutrient competition, and for decreased fecundity due to parasite infection of the gonads. Neither of these mechanisms appear to be functioning however, at least not to any appreciable degree.

Comparison of growth rates between non-infected and C. flabelliformis-infected L. stagnalis in Nicollet Lake was impossible, as all snails except the very young contained either sporocysts or metacercariae. Size comparisons between snails infected with either

of these two larval stages was possible however, and were made on five different occasions (Table A-6). Comparison of sample means on four out of five dates showed no statistical difference between sporocyst-infected and metacercarial-infected snails. Collections made on these dates were graphically displayed (Figures 11 and 13) and showed the size distributions of both groups of snails.

Although little difference in size was seen to exist between these two groups, L. stagnalis in Nicollet Lake regularly grew to a size infrequently described in the literature (Table 7). Wesenberg-Lund (1934) felt that trematodes were somehow responsible for enhancing growth in certain lymnaeids. Campbell (1973) likewise suggested that the rapid growth spurt seen in L. stagnalis in mid-summer may have resulted from infection with C. flabelliformis. While growth of this pulmonate certainly is influenced by the type of habitat and prevailing environmental conditions, parasitization may also play an important role. Considering that a large proportion of host tissues were replaced by either C. flabelliformis sporocysts or metacercariae, Wright's (1966) suggestion that pressure effects may be relieved by increases in shell capacity seem reasonable.

Effects of parasitism on reproduction were not studied, but the observation was made that both sporocyst and tetracotyle-infested snails did readily produce egg masses whenever brought into the laboratory. Considering that one hundred percent of snails of reproductive age were heavily infected with either of these two stages, reproduction and population stability of the host were either not seriously affected, or if so, were well compensated.

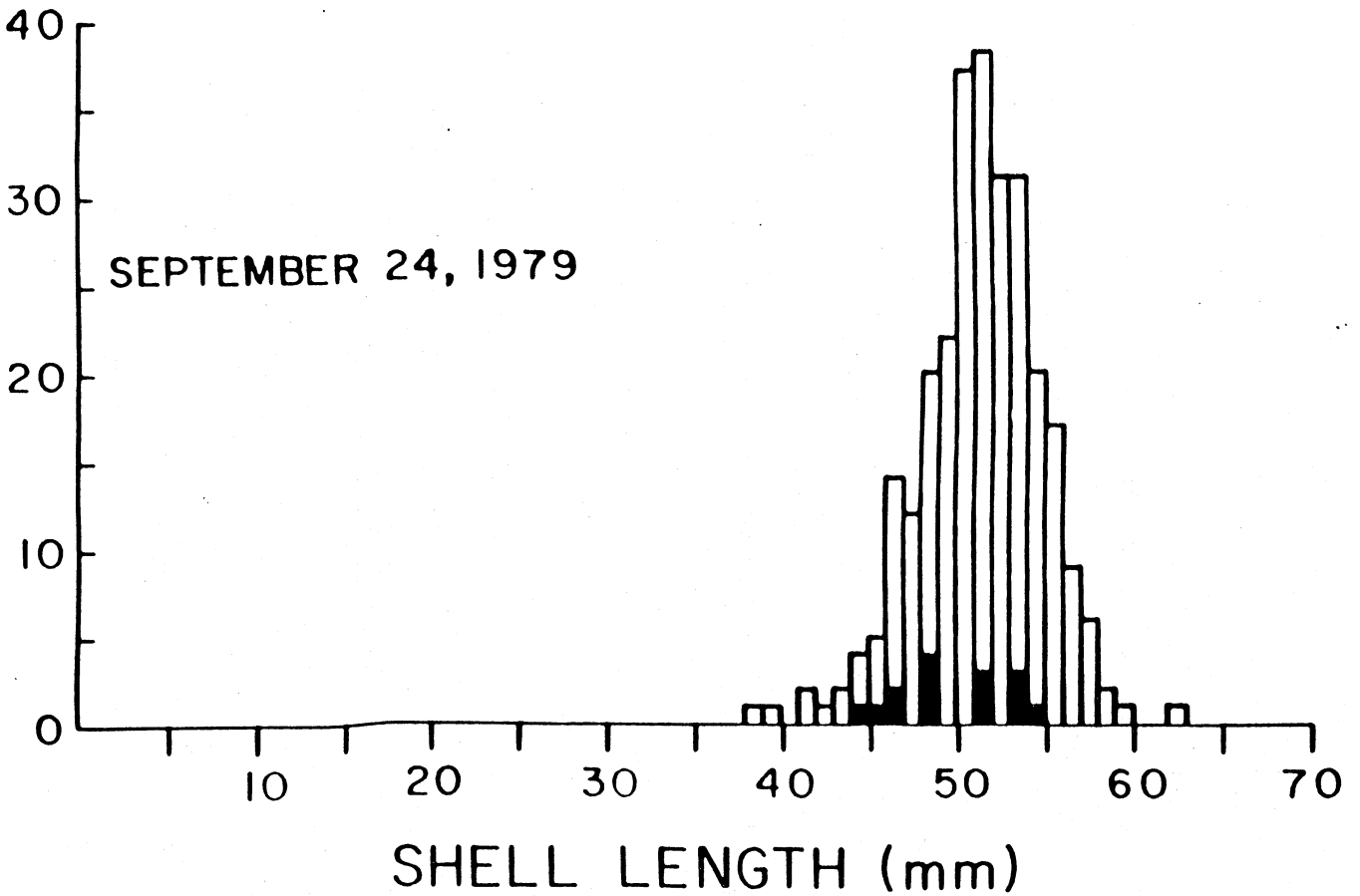

Figure 13. Altitude-frequency histogram of L. stagnalis infected with C. flabelliformis sporocyst (blackened squares) or sporocyst free (white squares) on September 24, 1979*.

*Comparison of sample means for size shows no statistical difference between the two groups,

Effects on Host and Non-Host Mortality:

Trematodes which utilize two molluscan hosts for intermediate stages of development pose a potential risk for overparasitization and increased mortality of the host. As discussed previously, various species of strigeid and echinostome trematodes have cercariae which actively penetrate both host and non-host molluscs. Significant mortality may occur when large numbers of these cercariae penetrate and migrate to the appropriate tissue or gland for further development.

Although metacercariae of C. flabelliformis only encyst in lymnaeid snails (and physid and planorbid snails if sporocysts of another trematode species are present) the cercariae used in this study did actively attach to and penetrate a variety of species in other molluscan families (Table 10). This list represents all species experimentally exposed to cercariae and implies an almost total lack of selectivity on the part of the parasite for its host. Campbell (1972) also found that several other non-lymnaeid species (H. trivolvis, P. gyrina, and Oxyloma retusa) attracted these cercariae, some more than others, suggesting that a type of chemoattraction was in operation.

The potential that cercarial penetration and metacercarial encystment have as mortality factors was examined by exposing various species of lymnaeid and planorbid snails to varying numbers of C. flabelliformis cercariae and comparing survival of these snails to that of uninfected controls (Figure 14; Table A-7). One group of B. glabrata was concurrently infected with S. mansoni. Upon cercarial

TABLE 10. Families and species of molluscs which were actively penetrated by cercariae of *Cotylurus flabelliformis* (present investigation).

Family Lymnaeidae

Lymnaea stagnalis
L. elodes (*Stagnicola palustris*)
L. reflexa (*S. exilis*)
Bulimnea megasoma
Acella haldemani

Family Physidae

Physa gyrina
Aplexa hypnorum

Family Planorbidae

Helisoma trivolvis
H. campanulata
Biomphalaria glabrata
Gyraulus parvus

Family Pleuroceridae

Pleurocera sp.

Family Valvatidae

Valvata tricarinata

Family Viviparidae

Viviparus georgianus

Family Unionidae

Lampsilis siliquoidea

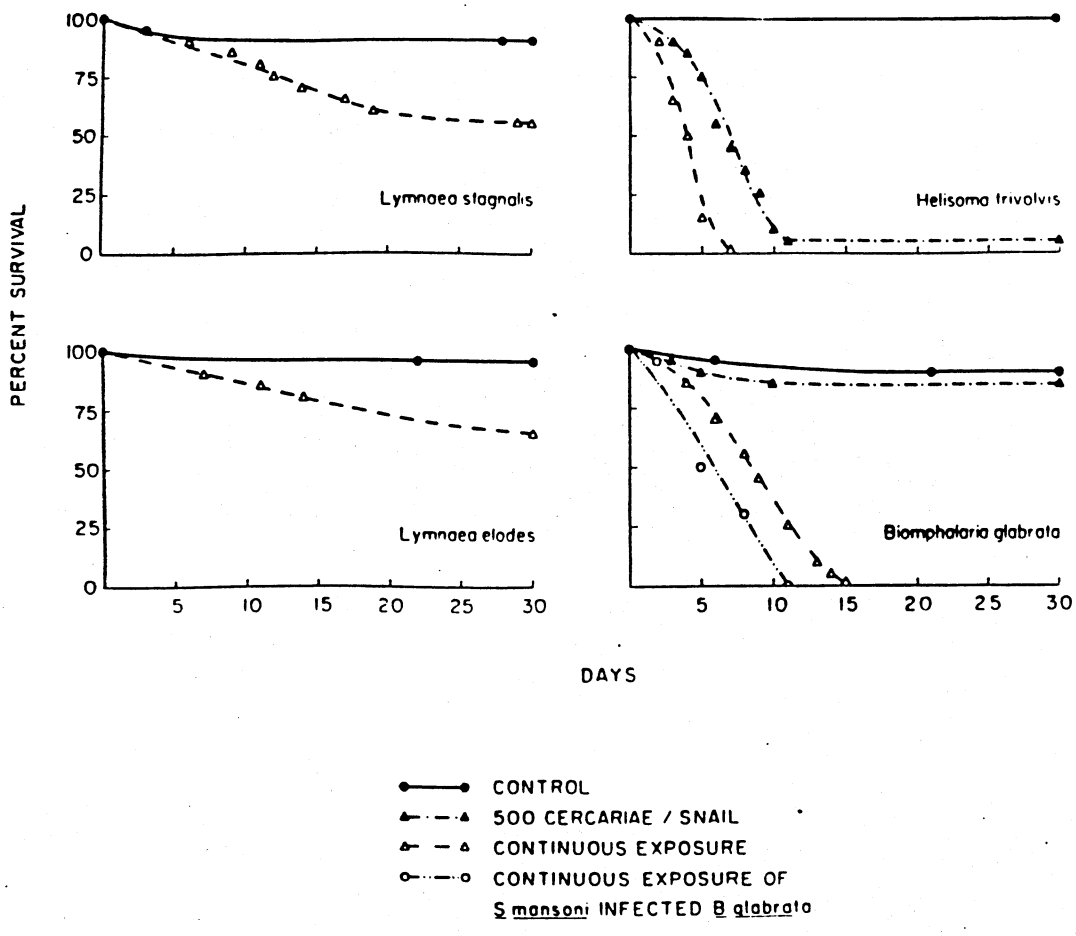

Figure 14. Survivorship curves for host (L. stagnalis and L. elodes) and non-host (H. trivolvis and B. glabrata) snails exposed to C. flabelliformis cercariae*.

*Each treatment group was composed of 20 snails. The one specimen of H. trivolvis surviving to 30 days was found to harbor large numbers of rediae and encysted metacercariae

penetration all snails became agitated, often withdrawing into their shells.

Both L. stagnalis and L. elodes showed similar mortality to a continuous exposure of C. flabelliformis cercariae (down to 55-65% survival after thirty days). H. trivolvis demonstrated high mortality to either treatment, with death occurring in seven to ten days. The one specimen of H. trivolvis that survived to day thirty was found to contain a redial generation which contained numerous hyperparasitic tetracotyles. B. glabrata did not show increased mortality to an exposure of 500 cercariae per snail as did H. trivolvis. As mentioned earlier however (Table 9), significant numbers of uninfected B. glabrata could be infected with C. flabelliformis metacercariae, whereas none were found in H. trivolvis unless sporocysts or rediae were present. Continuous exposure of the uninfected and S. mansoni-infected B. glabrata to the strigeid cercariae did result in the death of all snails by day fifteen.

These survivorship curves of snails exposed to varying numbers of C. flabelliformis cercariae demonstrate significant mortality for the two planorbid species when compared with the normal lymnaeid hosts. Considering the large number of tetracotyles (up to 3,000 or more) that L. stagnalis can harbor, mortality would seem to result from the penetration of large numbers of cercariae over a short period of time, causing much irritation to the host. Such large numbers however, would doubtfully be found in nature, suggesting that the importance of this process as a mortality factor is limited. Likewise, Kuris and Warren (1980) found that a high rate of cercarial

penetration with *E. liei* was necessary to cause significant mortality in *B. glabrata*. The use of cercarial penetration and early metacercarial activity as a means to aid population control of undesirable molluscan species remains a curiosity.

CONCLUSIONS

Habitat and Growth:

The Nicollet Lake habitat provides ideal conditions for the growth and reproduction of *L. stagnalis appressa*, including high calcium and alkalinity from the surrounding calcareous type of grey glacial drift. These conditions resulted in the strong growth and reproduction of this species.

Natural History of *L. stagnalis*:

Most *L. stagnalis* in Nicollet Lake have an annual life cycle (13-14 months) and overwinter once. Some may extend this up to 24 months and two breeding seasons by overwintering twice. Others may hatch, reproduce, and die in less than 6 months. This variation may be of significance in protecting the population from adverse environmental conditions.

Ecological Relationships of *C. flabelliformis*:

Variation from year to year in the numbers of sporocyst-infested snails observed in Nicollet Lake may reflect changes occurring in the habits of final hosts.

The numbers of tetracotyles recovered per snail increased at a predictable rate along with the size (age) of the snail host. This suggests that the number of tetracotyles per snail is limited more by the size of the individual than to the density of cercariae in the environment, once above a certain threshold value.

The role of overwintered adult snails is of vital importance in

maintaining a stable parasite population for several reasons: 1) they serve as a ready source of infective material for waterfowl passing through in the Spring migration, and 2) overwintered sporocyst-infested snails provide cercariae in the Spring from which a large percentage of tetracotyles in younger snails will ultimately develop.

Interpopulation variation was evident between this study and that of Campbell (1973b). Differences were apparent in the percentages of cercarial shedding snails, and times of peak infections. In Minnesota over one-half of the total number of tetracotyles per snail appeared by mid-July, whereas in Iowa the corresponding increase did not occur until mid-September. Intensity of infections also varied (greater in Minnesota) and probably reflected differences seen in the sporocyst infestation between the two habitats.

Although the life history of C. flabelliformis is well adapted to the migratory habits of the definitive hosts, the parasite population dynamics appear to be more strictly defined in terms of the mollusc's life cycle. The presence of definitive hosts Spring to Fall instead of only during migrations would probably not influence parasite population changes to any significant degree.

Intraspecific and Interspecific Trematode Antagonism:

The numbers of tetracotyles found in sporocyst-infested (C. flabelliformis) and sporocyst-free L. stagnalis were found to be statistically different when adjusted for snail size (age). The presence of schistosome sporocysts and some xiphidiocercariae-producing sporocysts similarly displayed a partial aversion to the establishment

of tetracotyle metacercariae.

Conversely, the planorbid snail H. trivolvis failed to develop a C.flbelliformis metacercarial infection unless sporocysts or rediae of other trematode species were present. S. mansoni-infected B. glabrata were likewise susceptible to tetracotyle development. Uninfected B. glabrata however also served as a satisfactory host for the establishment of tetracotyles, unlike H. trivolvis.

The presence of sporocysts (especially parasite mass in relation to total host mass) appears to be a limiting factor for tetracotyle development in lymnaeids, and a required factor for tetracotyle survival in planorbids.

Pathological Effects of C. flabelliformis on Growth and Mortality:

The large size routinely reached by L. stagnalis in Nicollet Lake (compared to other reports from different locales) suggested that parasitization may play an important role in growth. One hundred percent of sporocyst-free snails of reproductive age did contain large numbers of metacercariae. Comparisons between sporocyst-infected and sporocyst-free snails failed to reveal any consistent differences in size. Parasite-free snails were not available for comparison.

Unlike lymnaeid snails, significant mortality of planorbid snails occurred when exposed to large numbers of C. flabelliformis cercariae. Death occurred rapidly, resulting from the penetration and early migration in the host of large numbers of cercariae over a short time period. It is doubtful that the numbers and density of cercariae involved would be found in nature, suggesting that the importance of

this process as a mortality factor is limited.

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APPENDIX

TABLE A-1. Shell length measurements of *Lymnaea stagnalis* collected in Nicollet Lake, Clearwater County, Minnesota for the years 1975-1980. Water temperature measured at 10 cm is also noted.

DATE		SAMPLE SIZE	MEAN SHELL LENGTH (mm)	± 1 S.D.	(RANGE)	°C
1975						
5-15		98	4.0	1.0	(2-8)	-
		2	54.5	0.7	(54-55)	
5-27		100	6.9	4.1	(3-14)	-
	Total	200				
1976						
4-23	ice off	0	-	-	-	-
5-1		51	3.5	1.0	(2-7)	10.0
		18	48.6	3.2	(43-57)	
5-15		99	4.8	1.7	(2-9)	16.0
		29	49.1	2.6	(44-56)	
6-14		50	22.0	6.1	(13-34)	22.0
		25	52.0	2.9	(46-59)	
7-1		90	36.8	6.5	(13-50)	26.0
		9	54.4	2.7	(51-59)	
8-23		133	47.3	3.1	(36-56)	23.0
9-22		97	47.3	2.9	(36-54)	13.0
	Total	601				
1977						
4-20	ice off	0	-	-	-	-
4-23		1	49.0	-	-	11.0
4-30		18	3.2	0.9	(2-5)	15.0
		38	49.2	2.9	(43-54)	
5-14		28	5.5	2.1	(2-10)	22.0
		33	51.3	2.5	(46-58)	
5-30		33	19.7	4.2	(12-27)	22.0
		27	52.3	2.5	(47-58)	
6-18		91	35.0	4.7	(23-43)	23.0
		11	52.5	2.3	(49-57)	
6-30		58	44.4	6.6	(24-56)	24.5
		6	53.0	2.0	(51-56)	
7-11		68	48.5	5.1	(26-58)	23.0
7-30		109	52.2	4.0	(37-61)	24.0
8-15		113	54.8	3.1	(41-60)	20.0
9-1		11	2.0	0.4	(2-3)	16.5
		130	54.0	2.9	(48-62)	
9-17		103	55.3	3.3	(45-63)	17.5
10-16		56	54.8	3.2	(48-60)	7.0
	Total	934				
1978						
9-8		12	2.6	0.5	(2-3)	24.0
		186	47.9	3.2	(40-58)	
	Total	198				
1979						
9-24	Total	277	51.8	3.4	(39-63)	14.0
1980						
10-18	Total	4	48.4	5.2	(41-54)	7.0

TABLE A-2. Logistic Equation (Robertson, 1923; Plorin, unpublished) fitted to the 1976 and 1977 growth data on L. stagnalis appressa from Nicollet Lake.

	1976 Parameters			1977 Parameters		
	L_∞ = 47.46 mm Lo = 2.00 mm k = 0.0628/day			L_∞ = 53.77 mm Lo = 2.00 mm k = 0.0713/day		
DAY	CORRESPONDING MONTH/DAY	SHELL SIZE (mm)	GROWTH RATE (mm/wk)	CORRESPONDING MONTH/DAY	SHELL SIZE (mm)	GROWTH RATE (mm/wk)
0	4-29	2.00	-	4-23	2.00	-
7	5-5	3.03	1.03	4-30	3.22	1.22
14	5-12	4.55	1.52	5-7	5.10	1.88
21	5-19	6.70	2.15	5-14	7.92	2.82
28	5-26	9.65	2.95	5-21	11.91	3.99
35	6-2	13.47	3.82	5-28	17.15	5.24
42	6-9	18.07	4.60	6-4	23.42	6.27
49	6-16	23.18	5.11	6-11	30.10	6.68
56	6-23	28.34	5.16	6-18	36.39	6.29
63	6-30	33.08	4.74	6-25	41.68	5.29
70	7-7	37.07	3.99	7-1	45.72	4.09
77	7-14	40.20	3.13	7-8	48.58	2.86
84	7-21	42.52	2.32	7-15	50.49	1.91
91	7-28	44.15	1.63	7-22	51.73	1.24
98	8-4	45.27	1.12	7-29	52.51	0.78
105	8-11	46.03	0.77	8-5	53.00	0.49
112	8-18	46.53	0.50	8-12	53.30	0.30
119	8-25	46.85	0.32	8-19	53.48	0.18
126	9-1	47.07	0.22	8-26	53.59	0.11
133	9-8	47.21	0.14	9-2	53.66	0.07
140	9-15	47.30	0.09	9-9	53.70	0.04
147	9-22	47.35	0.05	9-16	53.73	0.03
154	9-29	47.39	0.04	9-23	53.74	0.01
161	10-6	47.42	0.03	9-30	53.75	0.01
168	10-13	47.43	0.01	10-7	53.76	0.01
175	10-20	47.44	0.01	10-14	53.76	0.00
182	10-27	47.45	0.01	10-21	53.76	0.00
189	11-3	47.45	0.00	10-28	53.77	0.00
196	11-10	47.46	0.01	11-4	53.77	0.00

TABLE A-2. (Continued)

Logistic Equation: $$L_t = \frac{Lo\ L_\infty}{Lo + (L_\infty - Lo)e^{-kt}}$$

L_t = size at age t

L_∞ = maximum size

Lo = starting size

k = rate of growth

Method of Finite Differences:

Measurements taken at equal time intervals with the reciprocals of successive sizes plotted against the following reciprocals to produce the line and estimates:

$$\frac{1}{L_t + T} = \frac{1}{L_t} e^{-kT} + \frac{(1-e^{-kT})}{L_\infty}$$

$\hat{L}_\infty$ = (1-slope)/intercept

$\hat{K}$ = -[ln(slope)]/T where T = time interval

Table A-3. Infection rates of *L. stagnalis* collected in Nicollet Lake for several commonly found cercarial types. Collections from 1976 to 1979 are included. All snails with a shell length greater than 28 mm harbored varying numbers of tetracotyle metacercariae. Those marked with an * were determined by individual dissection, otherwise individual overnite isolation in beakers with filtered lake or aged tap water was used.

Date	Mean Shell Length (mm)	*C. flabelliformis* (%)	Xiphidiocercariae (%)	*C. elvae* (%)	Unidentified (%)
5-1-76	48.6	1/18 (5.5)	1/18 (5.5)	-	-
	3.5	0/51 (0.0)	-	-	-
5-15-76	49.1	1/29 (3.4)	2/29 (6.9)*	-	-
	4.8	0/99 (0.0)	-	-	-
6-14-76	52.0	1/25 (4.0)	-	-	-
	22.0	4/50 (8.0)*	-	-	-
7-1-76	54.4	0/9 (0.0)	1/9 (11.1)	-	-
	36.8	1/90 (1.1)	-	-	-
9-22-76	46.9	18/97 (18.6)	2/97 (2.0)	-	3/97 (3.1)
4-30-77	3.2	0/18 (0.0)	-	-	-
	49.2	7/38 (18.4)	1/38 (2.6)	1/38 (2.6)	-
5-14-77	5.5	0/28 (0.0)	-	-	-
	51.3	8/33 (24.2)	2/33 (6.1)	-	1/33 (3.0)
5-30-77	19.7	0/33 (0.0)	-	-	-
	52.3	7/27 (25.9)	-	-	-
6-18-77	35.0	6/91 (6.6)	-	2/91 (2.2)	-
	52.5	6/11 (54.5)	1/11 (9.1)	-	-
6-30-77	44.4	0/58 (0.0)	3/58 (5.2)	-	-
	53.0	1/6 (16.7)	4/6 (66.7)	-	-
7-11-77	48.5	2/68 (2.9)	5/68 (7.4)*	1/68 (1.5)*	2/68 (2.9)*
7-30-77	52.2	2/109 (1.8)*	15/109 (13.8)*	-	2/109 (1.8)*
8-15-77	54.8	1/113 (0.9)*	2/113 (1.8)*	-	-
9-1-77	2.0	0/11 (0.0)	-	-	-
	54.0	1/130 (0.8)	11/130 (8.5)	-	-
9-17-77	55.3	3/103 (2.9)	20/103 (19.4)	-	1/103 (0.9)
10-16-77	54.8	1/56 (1.8)	12/56 (21.4)	-	-
5-18-78	54.0	2/61 (3.3)	10/61 (16.4)	2/61 (3.3)	-
9-24-79	51.8	15/262 (5.7)	1/262 (0.4)	-	-
TOTALS		88/1723 (5.1)	93/1723 (5.4)	6/1723 (0.3)	9/1723 (0.5)
OVERALL TOTAL		196/1723 (11.4%)			

Table A-4. Results from fitting the Logistic Equation to the 1977 L. stagnalis growth data from Nicollet Lake at seven day intervals. Metacercarial counts are calculated from the Least-Squares Regression line using snail size as predicted by the Logistic Equation.

Day	Corresponding Month/Day	Shell Size	% Max. Size	Growth Rate (mm/wk)	Log Number Metacer.	Number Metacer.	% Total Metacer.	Infection Rate (Tetracotyles/wk)
0	4-23	2.00	3.7	-	-	0	0.0	0
7	4-30	3.22	6.0	1.22	-	0	0.0	0
14	5-7	5.10	9.5	1.88	-	0	0.0	0
21	5-14	7.92	14.7	2.82	-	0	0.0	0
28	5-21	11.91	22.1	3.99	-	0	0.0	0
35	5-28	17.15	31.9	5.24	0.035	1	0.0	1
42	6-4	23.42	43.6	6.27	0.605	4	0.2	3
49	6-11	30.10	56.0	6.68	1.213	16	0.7	12
56	6-18	36.39	67.7	6.29	1.785	61	2.6	45
63	6-25	41.68	77.5	5.29	2.267	185	7.9	124
70	7-1	45.72	85.0	4.04	2.635	432	18.6	247
77	7-8	48.58	90.3	2.86	2.895	785	33.7	353
84	7-15	50.49	93.9	1.91	3.069	1172	50.3	387
91	7-22	51.73	96.2	1.24	3.181	1517	65.2	345
98	7-29	52.51	97.7	0.78	3.252	1786	76.7	269
105	8-5	53.00	98.6	0.49	3.297	1982	85.1	196
112	8-12	53.30	99.1	0.30	3.324	2109	90.6	127
119	8-19	53.48	99.5	0.18	3.341	2193	94.2	84
126	8-26	53.59	99.7	0.11	3.351	2244	96.4	51
133	9-2	53.66	99.8	0.07	3.357	2275	97.7	31
140	9-9	53.70	99.9	0.04	3.361	2296	98.6	21
147	9-16	53.73	99.9	0.03	3.363	2307	99.1	11
154	9-23	53.74	99.9	0.01	3.364	2312	99.3	5
161	9-30	53.75	100.0	0.01	3.365	2317	99.5	5
168	10-7	53.76	100.0	0.01	3.365	2323	99.8	6
175	10-14	53.76	100.0	0.00	3.365	2323	99.8	0
182	10-21	53.76	100.0	0.00	3.366	2323	99.8	0
189	10-28	53.77	100.0	0.01	3.367	2328	100.0	5
196	11-4	53.77	100.0	0.00	3.367	2328	100.0	0

TABLE A-5. Counts of tetracotyle metacercariae found in sporocyst-free L. stagnalis from Nicollet Lake on selected dates.

DATE	SAMPLE SIZE	MEAN SHELL LENGTH (mm)	MEAN NUMBER TETRACOTYLES	MEAN LOG TETRACOTYLES	± 1 S.D.	± 95% C.I.
April 30, 1977	18	49.2	1318	3.120	0.164	0.082
May 14, 1977	8	51.3	1109	3.045	0.280	0.234
May 30, 1977	6	22.2	2	0.326	0.370	0.388
	6	52.3	2065	3.315	0.196	0.206
June 18, 1977	19	35.4	37	1.565	0.632	0.305
June 30, 1977	8	44.4	285	2.455	0.530	0.443
September 24, 1979	17	53.2	2306	3.363	0.187	0.096

TABLE A-6. Effects of C. flabelliformis sporocyst infestation on the growth of L. stagnalis. Comparisons of shell length between sporocyst-free and sporocyst-infested L. stagnalis have been made for selected dates. All sporocyst-free snails do harbor large numbers of metacercariae.

DATE	SPOROCYST PRESENT	SAMPLE SIZE	MEAN SHELL LENGTH ± 1 S.D.	t value	n
4/30/77	no	31	49.3 3.1		
	yes	7	48.6 2.2	0.564	NS
5/14/77	no	25	50.7 2.1		
	yes	8	53.0 2.8	2.486	< 0.025
5/30/77	no	20	51.9 2.1		
	yes	7	53.4 3.2	1.417	NS
6/18/77	no	85	34.9 4.7		
	yes	6	37.2 4.8	1.157	NS
	no	5	52.6 2.9		
	yes	6	52.5 2.1	0.066	NS
9/24/79	no	262	51.9 3.4		
	yes	15	50.3 3.2	1.777	NS

TABLE A-7. Mortality of host (*L. stagnalis* and *L. elodes*) and non-host (*H. trivolvis* and *B. glabrata*) snails when exposed to varying numbers of *C. flabelliformis* cercariae. 100 or 500 cercariae/snail implies a single exposure on day 0. Continuous exposure indicates that an individual *L. stagnalis* shedding *C. flabelliformis* cercariae has been placed with the treatment group (20 snails) and remains throughout the experiment.

TREATMENT GROUP	DAY	NUMBER SURVIVING	PERCENT SURVIVAL
L. stagnalis - control	0	20	100
	3	19	95
	28	18	90
	30	18	90
L. stagnalis - continuous exposure	0	20	100
	6	18	90
	9	17	85
	11	16	80
	12	15	75
	14	14	70
	17	13	65
	19	12	60
	29	11	55
	30	11	55
L. elodes - control	0	20	100
	22	19	95
	30	19	95
L. elodes - continuous exposure	0	20	100
	7	18	90
	11	17	85
	14	16	80
	30	13	65
H. trivolvis - control	0	20	100
	30	20	100
H. trivolvis - 100 cercariae/snail	0	20	100
	30	20	100
H. trivolvis - 500 cercariae/snail	0	20	100
	3	18	90
	4	17	85
	5	15	75
	6	11	55
	7	9	45
	8	7	35
	9	5	25
	10	2	10
	11	1	5
	30	1	5

TABLE A-7. (Continued)

TREATMENT GROUP	DAY	NUMBER SURVIVING	PERCENT SURVIVAL
H. trivolvis - continuous exposure	0	20	100
	2	18	90
	3	13	65
	4	10	50
	5	3	15
	7	0	0
B. glabrata - control	0	20	100
	6	19	95
	21	18	90
	30	18	90
B. glabrata - 100 cercariae/snail	0	20	100
	30	20	100
B. glabrata - 500 cercariae/snail	0	20	100
	3	19	95
	5	18	90
	10	17	85
	30	17	85
B. glabrata - continuous exposure	0	20	100
	4	17	85
	6	14	70
	8	11	55
	9	9	45
	11	5	25
	13	2	10
	14	1	5
	15	0	0
B. glabrata - *S. mansoni* infected continuous exposure	0	20	100
	2	18	90
	5	10	50
	8	6	30
	11	0	0